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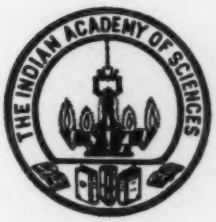

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MEXICO

ADDITIONS TO THE FUNGI OF MADRAS—XVII

By T. S. Ramakrishnan and N. V. Sundaram

Received January 8, 1955

Plasmopara wildemaniana P. Henn.

P. A. Saccardo, *Syll. Fung.*, **21**, 861.

On living leaves of *Justicia simplex* D. Don. (Acanthaceæ), Ambalavayal (Wynaad), 3–9–1954, N. V. Sundaram.

The imperfect stage alone was present. The same fungus has been previously recorded on *Peristrophe bicalyculata* from this State.

Catacauma microcentum (B. and Br.), Theiss. and Syd.

F. Theissen and H. Sydow., *Ann. Mycol.*, 1915, **13**, 384.

On living leaves of *Artocarpus hirsuta* Lamk. (Moraceæ), Mangalore, 12–11–1954, T. S. Ramakrishnan.

The stromata are amphigenous and form isolated or confluent crusts.

Elsinoe fawcetti Bitancourt and Jenkins.

A. A. Bitancourt and A. E. Jenkins, *Phytopath.*, 1936, **26**, 393–96.

On living leaves and fruits of *Citrus paradisi* (Rutaceæ), Kallar, 10–11–1953, T. S. Ramakrishnan; on leaves and fruits of *C. sinensis*, Tudiyalur (Coimbatore) and Kodur, 15–10–1952, T. S. Ramakrishnan.

The scab of *Citrus* has been observed in different parts of the State in recent years. The incidence is however confined to the two species recorded above. The imperfect stage—*Sphaceloma fawcetti* Jenkins—alone has been noticed. The leaves and fruits are infected. The disease on *C. sinensis* has been kept under control by repeated sprayings of Bordeaux mixture.

Guignardia bidwellii (Ell.) Viala and Rav.

On fruits of *Vitis vinifera* L. (Ampelideæ), Ganguvarapatty (Madura District), 5–8–1953, N. V. Sundaram.

The black rot of grapes has been recorded for the first time from this State. The pycnidial state was produced in abundance on the berries and their stalks. The disease causes serious damage to some exotic purple-fruited varieties especially when rains are received at the fruiting period.

The local green variety is much less infected. The disease becomes evident when the fruits are half ripe and entire bunches rot and become blackened. The results of the experiments to control the disease showed that the disease could be easily kept in check by spraying the bunches with Cupravit OB 21 or Bordeaux mixture, once after fruit set and again after an interval of a fortnight. More sprayings are necessary if the weather continues to be humid.

Mycosphærella agapanthi-umbellati nov. sp.

Syn. *M. agapanthi* Ramak. T. S. and K. *Proc. Indian Acad. Sci.*, 1950, **32 B**, 98.

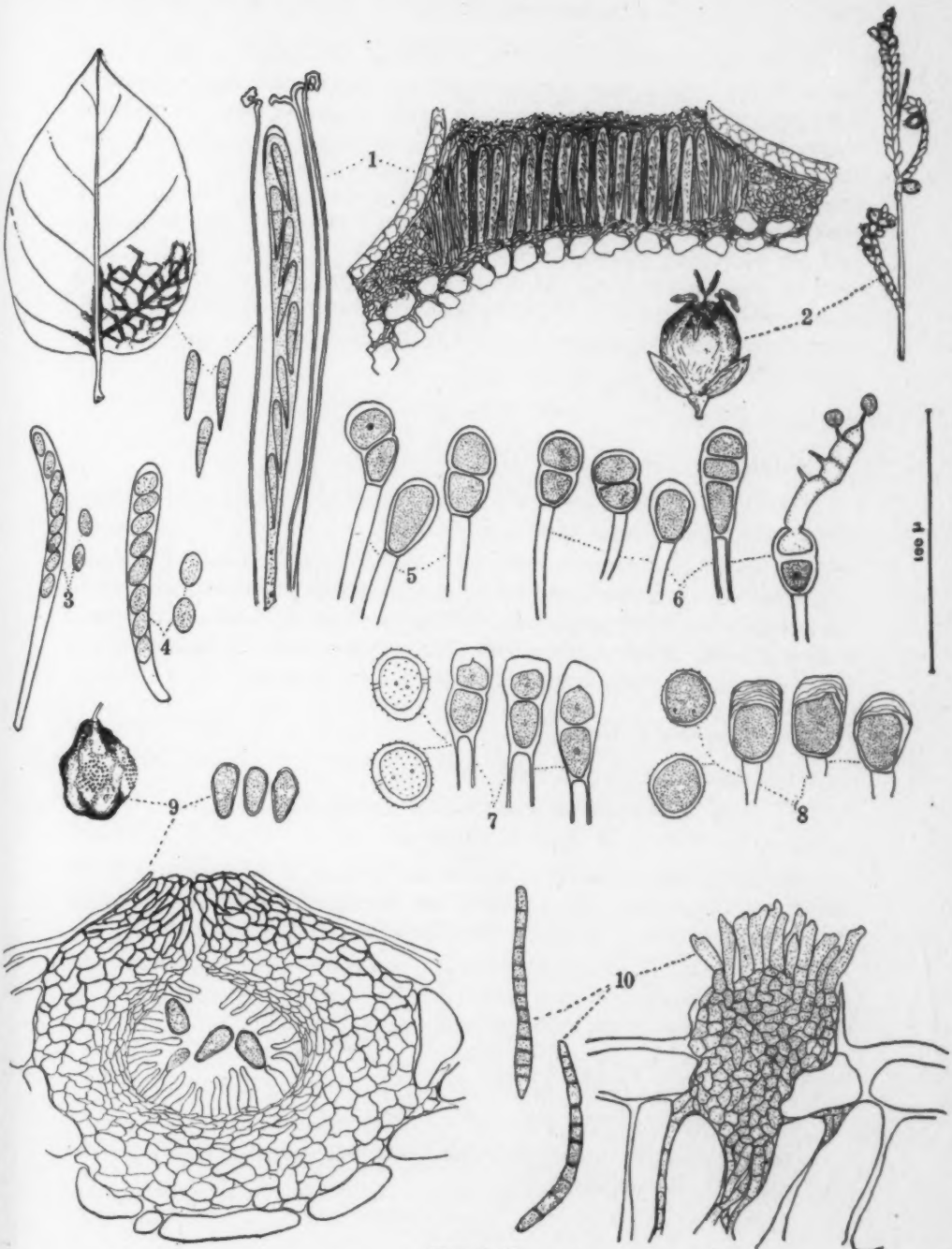
Ramakrishnan, T. S. and K. have described *M. agapanthi* on *Agapanthus umbellatus*. This specific name for the pathogen has been used earlier by Lindau (in Engler and Prantl., *Naturl. Pflanzenfam.* 1 Teil, 1 Abt., 1897), p. 427). But the fungus recorded by them is different. Therefore the specific name has to be changed as mentioned above.

Hypoderma leschenaultiæ nov. sp.

Patches foliicolous, irregular, brown, mostly epiphyllous; apothecia invariably epiphyllous, dark brown, strap shaped, branching profusely to simulate a reticulate appearance, mostly along the midrib and the lateral veins, intraepidermal and later erumpent, dehiscing longitudinally by a long split all along the length, up to 2 cm. long and 1 mm. broad; asci hyaline, clavate, 8-spored, $151 \times 12\,\mu$ (117–189 × 10·5–16), paraphysate, paraphyses hyaline, longer than the asci, filiform, often hooked or branched at the apex; ascospores clavate, broader at the apex and tapering towards the base, apex rounded, obliquely arranged, hyaline, becoming 1-septate at maturity only, $31 \times 3 \cdot 5\,\mu$ (25–37 × 3–4·5).

Maculæ foliicolæ, irregulares, brunneæ, ut plurimum epiphyllæ, apothecia semper epiphylla, fusce brunnea, elongata, ita profuse ramosa ut simulent reticulationem, ut plurimum ad nervos medium et laterales, intraepidermalia et tandem erumpentia, dehiscentia longitudinaliter per totam longitudinem, usque 2 cm. longa 1 mm. lata; asci hyalini, clavati, octospori, $151 \times 12\mu$ (117–189 × 10·5–16), paraphysati, paraphysibus hyalinis, asco longioribus, filiformibus, sæpe curvatis vel ramosis ad apicem; ascosporæ clavatæ, latiores ad apicem, fastigatæ ad basim, apice rotundato, oblique dispositæ, hyalinæ evadentes uniseptatæ tantum ad maturitatem, $31 \times 3 \cdot 5\,\mu$ (25–37 × 3–4·5).

On living leaves of *Lonicera leshenaultii* Wall. (Caprifoliaceæ), Ootacamund, 10–5–1954, T. S. Ramakrishnan.



FIGS. 1–10

Brown discoloured patches are formed on the upper surface of the leaves in the early stages. On these patches the characteristic reticulately branched ribbon-like apothecia can be easily distinguished. In advanced stages the discolouration extends to the lower surface of the leaves also. But the apothecia are usually observed on the upper surface only. Very rarely incipent development of the apothecia was noticed on the corresponding lower surface also. The upper surface is brown. The hypothecium is hyaline and can be readily differentiated. The parphyses are filiform and straight, bent or sometimes branched at the apex. The apices are closely entangled and do not separate readily. The ascospore is one-celled in the early stages. Even at maturity the septum can be distinguished only in some under the oil-immersion lens.

Phyllachora aliena Syd.

Sydow, H. and P., *Ann. Mycol.*, 1912, **10**, 407.

On living leaves of *Memecylon deccanense* (Myrtaceæ), Mangalore, 15–11–1954, T. S. Ramakrishnan.

Circular hypertrophied spots are formed on the leaves. These are yellow or rose coloured in the initial stages. Later the colour fades when the stromata develop epiphyllously. These are dark and form raised dome-shaped growths on the upper surface with corresponding depressions on the lower surface. In some cases the stromata form a raised ring.

Phyllachora minuta P. Henn.

Hennings, P., *Hedwigia*, 1902, **41**, 143.

On living leaves of *Hibiscus tiliaceus* L. (Malvaceæ), Alwaye, (T.-C. State), 8–12–1954, T. S. Ramakrishnan.

Black stromata are visible on both sides of the leaf in the centre of reddish brown irregular spots. The perithecia are developed deepseated occupying the entire thickness of the leaf. The paraphyses break down early.

Older perithecia are readily invaded by *Fusarium* sp. which results in their disorganisation. A pink growth develops over the stromata and on these the conidia of the hyperparasite are produced.

Ustilago trichophora (Link) Kunze

P. A. Saccardo, *Syll. Fung.*, 1888, **7**, 462.

On the ovaries of *Paspalidium geminatum* Stapf (Gramineæ), Mangalore, 10–11–1954, T. S. Ramakrishnan.

Infection is confined to some of the spikelets in the inflorescence. The sorus is enlarged 4 to 6 times the size of the normal grain. It has usually 3 conical projections at the apex and on these the anthers are borne. From the centre arise the two stigmatic branches.

Phakopsora vitis Syd.

P. Sydow, *Hedwigia*, 1899, **38**, 141.

E. J. Butler, *Ann. Mycol.*, 1912, **10**, 153–58.

On living leaves of *Vitis vinifera* L. (Ampelideæ), Kallar (Coimbatore), 15–11–1952; on *Ampelocissus arnottiana* Planch. (Ampelideæ), Walayar (Malabar), 25–12–1954, T. S. Ramakrishnan and N. V. Sundaram.

This rust is prevalent on the cultivated grapes in Coimbatore, Kallar and Ketti (Nilgiris) affecting mainly the blue grapes. The more widely cultivated green variety is not infected. The uredia form bright orange powdery sori on the lower surface of the leaves, often covering the entire surface. The affected leaves turn prematurely yellow and are shed. Besides infecting grapes the same rust is also prevalent on *Cissus* sp. and *Ampelocissus arnottiana* in Walayar and Coimbatore. The incidence of the rust on the grapes occurs during the cold months alone. The uredial state alone was present on all these.

Puccinia elytrariæ P. Henn.

Sydow, P. and H., *Monogr. Ured.*, 1904, **1**, 232.

On living leaves of *Justicia betonica* L. (Acanthaceæ), Sultan's Battery (Malabar), 3–9–1954, N. V. Sundaram.

The telia are hypophyllous and foliicolous forming compact dark brown erumpent structures of varying sizes. Numerous mesospores are found along with the normal 2-celled teliospores. Rarely 3-celled telispores are also noticed.

Puccinia iridis (D.C.) Wallr.

P. A. Saccardo, *Syll. Fung.*, 1888, **7**, 657.

On living leaves of *Iris germanica* (Iridæ), Sims' Park, Coonoor, 4–10–1954, N. V. Sundaram.

Both uredia and telia are present on both sides of the leaves. The uredia were severely infected by *Darluca filum*.

Puccinia tiliæfolia sp. nov.

Rust spots indefinite, amphigenous, brown; telia hypophyllous, gregarious, pulvinate, erumpent, brown, minute; teliospores 2-celled, elliptic, oblong or obovate, apex rounded, sometimes thickened (up to 4·5 μ), slightly constricted at the septum, brown, 27 × 16 μ (19–43 × 12–19), pedicel persistent, subhyaline, up to 65 μ long; mesospores are numerous, obovate, 19–25 × 12–19 μ; 3-celled spores are also seen rarely.

Maculæ rubiginosæ indefinitæ, amphigenæ, brunneæ; telia hypophylla gregaria, pulvinata, erumpentia, brunnea, minuta; teiliosporæ bicellulatæ, ellipticæ, oblongæ vel obovatæ, apice rotundato crasso (usque 4·5 μ), tenuiter constrictæ ad septum, brunneæ, 27 × 16 μ (19–43 × 12–19), pedicellis persistentibus, subhyalinæ usque ad 65 μ longæ; mesosporæ plurimæ, 19–25 × 12–19 μ; ter-cellulatæ sporæ visæ sed raro.

On living leaves of *Grewia tiliæfolia* Vahl. (Tiliaceæ), Ambalavayal (Malabar), 3-9-1954, N. V. Sundaram.

Several telia are formed close together forming compact groups. The telial stage alone was present. The teliospores germinate readily on maturity. The basidiospores are globose and are borne on sterigmata.

Uromyces anotis-monospermæ sp. nov.

Uredia hypophyllous, minute erumpent, cinnamon to chestnut brown; urediospores subglobose or obovate, ochraceous brown, echinulate, 25 × 19 μ (22–31 × 16–22), germpores 2 or 3, subequatorial; telia mixed with uredia and similar in appearance; teliospores 1-celled, elliptic, oblong or obovate, smooth, apex rounded, flattened or obtuse, very much thickened (up to 16 μ) yellowish brown, 34 × 19 μ (25–45 × 16–22), pedicellate, pedicel subhyaline.

Uredia hypophylla, minuta, erumpentia, cinnamomo vel castaneo brunnea; uredosporæ subglobosæ vel obovatæ, ochraceo-brunneæ, echinulatæ, 25 × 19 μ (22–31 × 16–22), germinationis poris binis vel ternis, subequatorialibus; telia *cum* urediis mixa, eisque similia aspectu; teliosporæ semel cellulatæ, ellipticæ, oblongæ vel obovatæ, leves apice rotundato, vel complanato, vel obtuso et crassissimo (usque ad 16 μ), luteo-brunneæ, 34 × 19 μ (25–45 × 16–22), pedicellatæ, pedicellis subhyalinis.

On living leaves of *Anotis monosperma* Benth. (Rubiaceæ), Nanjanad (Nilgiris), 3-11-1954, N. V. Sundaram.

The germpores in the urediospores are usually two in number but some spores have 3 germpores also. These are located below the middle, half way to the base. The teliospores are much thickened at the apex and

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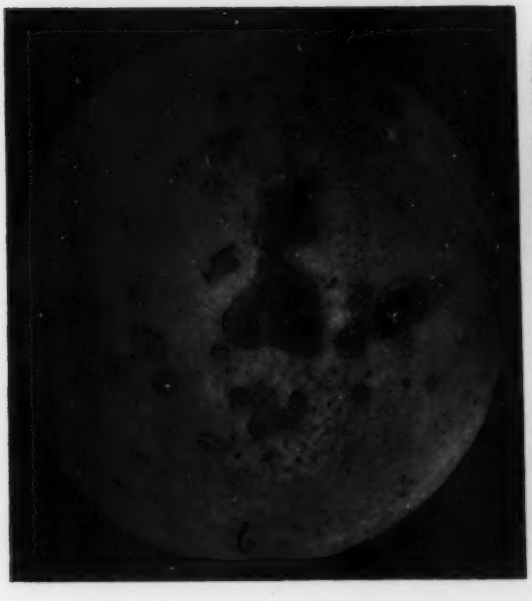


lamellations are evident. The lateral walls are very thin. This rust differs from *U. anotidis* Petch in not possessing æcia and in the teliospores not being crusted or incised.

Cercospora gliricidiæ Syd.

Sydow, *Philipp. J. Sci.*, 1913, **8**, 283.

On living leaves of *Gliricidia maculata* L. (Papilionatæ), Pattambi, 2–12–1954, N. V. Sundaram.

The fungus causes indefinite yellowish spots visible on both sides of the leaves. On both sides of the spots black stromata occur as minute dots. The conidiophores are brown, flexuous, with one or two septa and geniculate apex. The conidia are fuliginous elongate and measuring 28–90×4–8 μ. Three to nine septa may be present. Chupp is of opinion that this may belong to *Corynuem*.

Cercospora jasminicola Muller and Chupp.

C. Chupp, *Monogr.* of fungus genus *Cercospora*, 1954, 416.

On living leaves of *Jasminum grandiflorum* L. (Oleaceæ), 15–12–1954, Coimbatore, T. S. Ramakrishnan.

The fungus causes reddish brown circular or irregular amphigenous spots of varying sizes on the leaflets. In rainy weather the infection spreads rapidly resulting in the withering of the foliage accompanied by defoliation. The disease is prevalent from July to January in Coimbatore.

We wish to express our grateful thanks to Rev. Fr. H. Santhapau for the Latin translation and to the Professor of Botany, Agricultural College, Coimbatore, for the identification of some of the host plants.

Explanation of Plate

Plate IX

a. Scab of *Citrus* on leaves of *C. paradisi; b*. Scab on fruits of *C. paradisi; c*. Scab on fruits of *C. sinensis*.

Explanation of Text-Figures

Figs. 1–10. Fig. 1. *Hypoderma leschenaultiæ*, diseased leaf, section of apothecium (diagrammatic), ascus and ascospores. Fig. 2. *Ustilago trichophora*, infected panicle and a single sorus (enlarged). Fig. 3. *Phyllachora aliena*, ascus and ascospores. Fig. 4. *Phyllachora minuta*, ascus and ascospores. Fig. 5. *Puccinia elytrariæ*, teliospores and mesospore. Fig. 6. *Puccinia tiliæfolia*, teliospores, mesospore and germinating teliospore. Fig. 7. *Puccinia iridis*, urediospores and teliospores. Fig. 8. *Uromyces anotis-monospermæ*, urediospores and teliospores. Fig. 9. *Guignardia bidwellii*, infected berry, pycnidium and pycnidiospores. Fig. 10. *Cercospora gliricidiæ*, section through a cluster of conidiophores and conidia.

CYTOGENETIC STUDIES OF APOMIXIS IN *PENNISETUM*

Pennisetum clandestinum Hochst.*

By K. N. Narayan

(*Department of Botany, Central College, Bangalore*)

Production of morphologically uniform seedling progenies by plants containing aneuploid chromosome numbers has been the basis on which apomixis is postulated in plants. In *Pennisetum*, it has been shown that species with aneuploid chromosome numbers, *viz.*, *P. villosum* with $2n = 45$, *P. setaceum* with $2n = 27$ and *P. orientale* with $2n = 27$, are apomictic (Narayan, 1951).

P. clandestinum Hochst. ex Chiov., with $2n = 36$ is a native of Africa (Hitchcock, 1950), and consists of two varieties essentially comparable to those described by Edwards (1937) as *Rongai* and *Kabete*. Both the varieties have the same aneuploid chromosome numbers $2n = 36$. However, they differ from each other in certain important features, *viz.*, the *Kabete* variety (Plate X, Fig. 2) forms good pollen; is perfectly fertile and can be easily recognised by its prominently exserted anthers, whereas in the *Rongai* variety (Plate X, Fig. 1) the stamens are not exserted and no pollen is formed although seeds are set when pollinated.

The present investigation was taken up, firstly, to ascertain whether apomictic reproduction in *Pennisetum* was limited to species with aneuploid chromosomes and secondly, to find out the genetic basis of the differences (described above) that exist between the two named varieties.

Material and Methods

Both the varieties were found being grown as lawn grasses in the residential district at Berkeley. Material for the study of microsporogenesis was fixed in 3 : 1 absolute alcohol to acetic acid. Smears were made in aceto-carmine. Slides were made permanent whenever necessary according to the method outlined by McClintock (1929). Pollen fertility was tested by examining a random sample of 1100 pollen grains stained with cotton blue in lactophenol. The stainability of pollen was taken as the criterion of viability.

* Part of the thesis submitted in partial fulfilment for the Degree of Doctor of Philosophy in the University of California, California.

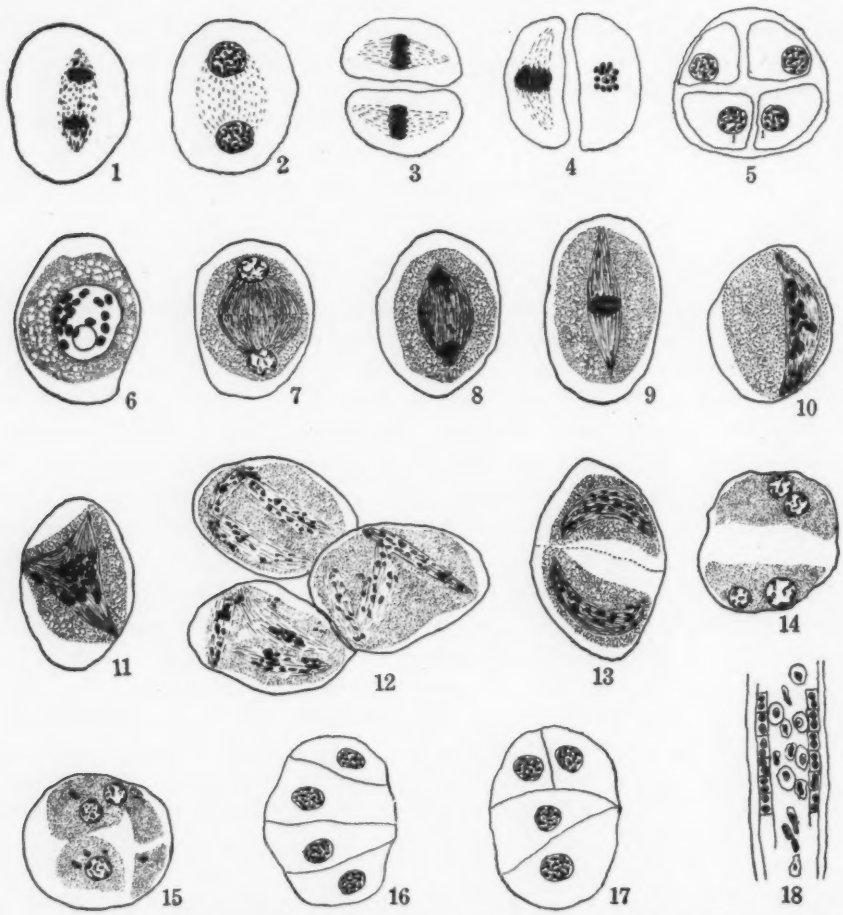
Material for embryological study was fixed in Randolph's Craf fluid or in acetic alcohol. Dehydration and embedding were carried out in the usual manner using butyl alcohol as the clearing agent. Sections were cut at 10–16 μ and stained in Heidenhains's stain. Slides of material fixed in acetic alcohol were treated with 1% chromic acid for about 6 hours before staining.

Root tips for somatic chromosomes were fixed in Craf. Sections were cut at 10 μ and stained in Stockwell's.

Microsporogenesis

P. clandestinum var. *Kabete*.—The various meiotic stages of microsporogenesis in this variety are essentially normal. At metaphase I, 18 bivalents are regularly formed (Plate X, Fig. 6). At first anaphase a few laggards and precocious chromosomes are found scattered on the spindle (Fig. 1). However, at the end of the first division all the laggards are included in the dyad nuclei (Fig. 2) and no micronuclei are formed. The second division is normal, the spindles being either parallel (Fig. 3) or at right angles (Fig. 4) to each other. Regular tetrads (Fig. 5) are formed and 80% of the pollen is viable.

In *P. clandestinum* var. *Rongai*.—Meiosis starts regularly as in the *Kabete*, and at diakinesis 18 bivalents are formed (Fig. 6). This regularity might continue and result in the completion of the first division (Fig. 7). Although laggards (Fig. 6) and precocious chromosomes (Fig. 9) are occasionally seen they do not seem to be of any consequence in contributing to the degeneration of the pollen mother cells. The aberrations that are responsible for the degeneration of the pollen originate at different stages of pollen mother cell development. Three major causes of pollen degeneration may be recognised: (i) those affecting the spindles; (ii) those affecting cytokinesis, and (iii) those affecting the orientation of microspores within a tetrad. A very characteristic feature of the spindle aberration is the presence of bent, twisted, and tripolar spindles, and of irregularly scattered chromosomes strewn all over the spindle (Figs. 10, 11). The tripolar spindle may lead to the formation of triads. Absence of cytokinesis and the institution of the next division of these triads will lead to the situation in Fig. 12. Spindle irregularities might affect the second division spindles also (Fig. 13), and nuclear division may or may not be followed by cytokinesis (Fig. 14). Often, the nuclear divisions followed by cytokinesis result in the formation of 4 cells but the orientation of the spindles and wall formation might be so irregular as to give rise to a linear tetrad (Fig. 16) or a T-shaped tetrad (Fig. 17). These irregularities might lead to further disturbances in chromosomes resulting in fragmentation (Figs. 12, 13). Degeneration of pollen mother cells in the



FIGS. 1–18. Microsporogenesis in *Pennisetum*. Figs. 1–5. *P. clandestinum* var. *Kabete*. Fig. 1. Late anaphase showing slight irregularity of precocious and lagging chromosomes, ×900. Fig. 2. Telophase I and dyad formation, ×900. Fig. 3. Second division in which spindles are parallel to each other, ×900. Fig. 4. Second division, spindles at right angles to each other ×900. Fig. 5. Normal tetrad formation, ×900. Figs. 6–18. *P. clandestinum* var. *Rongai*. Fig. 6. Diakinesis showing 18 bivalents, ×900. Fig. 7. Telophase I showing dyad formation, ×900. Fig. 8. Late anaphase showing laggards, ×900. Fig. 9. Metaphase I with precocious chromosomes, ×900. Fig. 10. Bent spindle with chromosomes strewn all over the spindles, ×900. Fig. 11. Tripolar spindles, ×900. Fig. 12. Three pollen mother cells each with three irregular spindles, ×900. Fig. 13. Irregularity of spindles in II division, ×900. Fig. 14. Absence of cytokinesis in II division, ×900. Fig. 15. Polyad formation with micronuclei, ×900. Fig. 16. Linear tetrad, ×900. Fig. 17. T-shaped tetrad, ×900. Fig. 18. Portion of an anther locule showing degeneration of pollen mother cells in earlier stages; tapetum consists of binucleate cells and appears to be healthy, ×900.

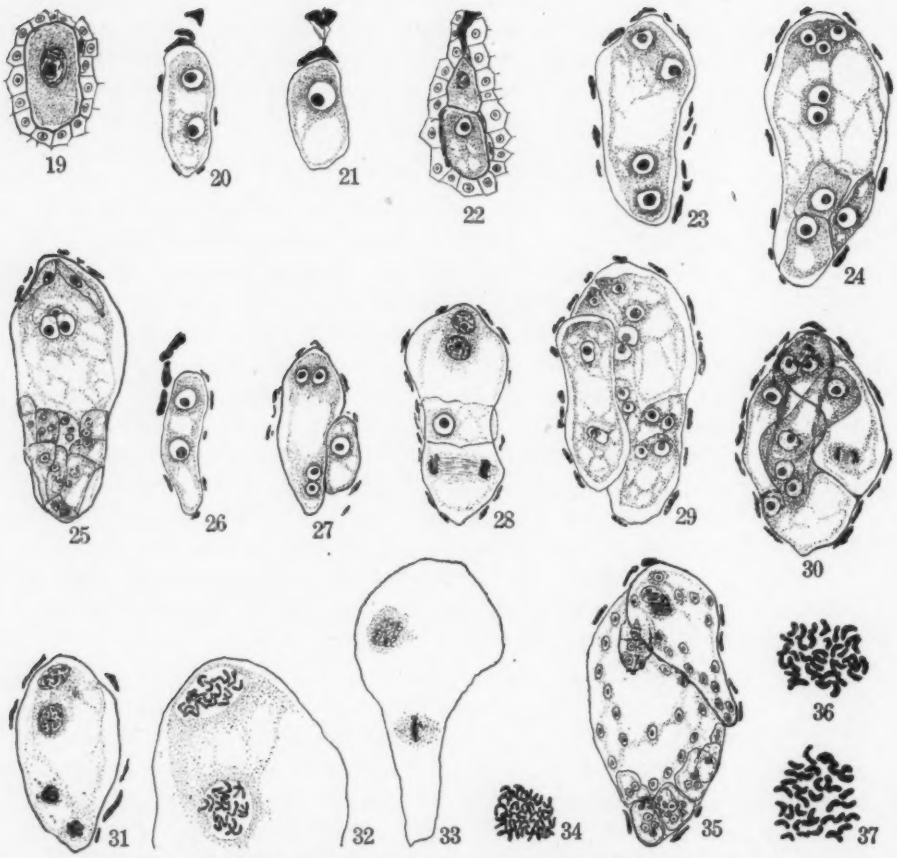
early prophasic stages of meiosis is often observed (Fig. 18). The tapetum is binucleate and healthy and does not seem to contribute to the degeneration. Whatever might be the stage at which these irregularities arise, the ultimate result is the same, *i.e.*, a complete degeneration of pollen mother cells. The anthers are not at all exserted.

Megasporogenesis and Embryo Sac Formation

The sexual cycle of the embryo sac formation is normal in both varieties. The archesporium consists of a single embryo sac mother cell (Fig. 19) of hypodermal origin which usually undergoes the usual reduction division resulting in the formation of the linear tetrad (Fig. 20). Sometimes a triad may be formed by the micropylar dyad degenerating without division (Fig. 22). Whenever the chalazal megaspore develops into an embryo sac its nucleus undergoes the 3 usual meiotic divisions resulting in the formation of an 8-nucleate embryo sac (Figs. 20, 23, 24) consisting of a micropylar and a chalazal quartet of nuclei. From the micropylar quartet the egg apparatus is organised. The synergid nuclei are emphemeral. The two polars lie close together. The antipodals consist of 3 cells in the earlier stages (Fig. 24) but later on they undergo 1 or 2 divisions giving rise to about 6–12 cells (Figs. 25–29). The nuclei in each of these cells undergo further divisions resulting in 2 to 6 nuclei in each cell. All the nuclei in a cell lie together in groups and the cells are highly vacuolated (Fig. 25).

Aposporic embryo sacs.—In a majority of ovules both aposporic and haploid embryo sacs are formed simultaneously. The correct identification of an embryo sac as either aposporic or haploid is very difficult unless the embryo sac sectioned is in the proper stage of development. When several embryo sacs are present in one ovule and when they are in their proper stage of development as in Plate X, Fig. 4 the haploid ones may sometimes be recognised by their relatively small nuclei. The developing megaspore with the 3 degenerating micropylar megaspores forming a T-shaped tetrad (Fig. 21) is sexual in origin, while the binucleate embryo sac with the 4 masses of material above, representing 4 degenerating megaspores (Fig. 26) appears to be aposporic.

Although aposporic initials usually arise either during or immediately after meiosis of the embryo sac mother cell, their origin is not limited always to these stages. They may arise at comparatively later stages of the haploid embryo sacs as in Fig. 27. The difference in size of the nuclei of the two embryo sacs in question strongly indicates that the 4-nucleate embryo sac is haploid and the uninucleate one aposporic. This figure further illustrates a late initiation of apospory. Figure 28 with a 4-nucleate embryo



FIGS. 19–37. Megasporogenesis and embryo sac formation in *P. clandestinum* var. *Kabete* and var. *Rongai*. Fig. 19. Megaspore mother cell (*Kabete*), ×420. Fig. 20. A two-nucleate embryo sac (*Rongai*) with three degenerating megaspores, ×420. Fig. 21. A T-shaped tetrad (*Rongai*), ×420. Fig. 22. A triad (*Kabete*) showing degeneration of micropylar dyad and megaspore the chalazal megaspore is enlarging and vacuolating, ×420. Fig. 23. A 4-nucleate embryo sac (*Rongai*), ×420. Fig. 24. Eight-nucleate embryo sac (*Rongai*), ×420. Fig. 25. A mature embryo sac (*Kabete*) showing multicellular cœnocytic and vacuolate antipodals, ×420. Fig. 26. A two-nucleate embryo sac (*Rongai*) with all four megaspores of linear tetrad degenerating, ×420. Fig. 27. A four-nucleate haploid embryo sac enlarging and vacuolating (*Kabete*), ×420. Fig. 28. A four-nucleate haploid embryo sac with an aposporic initial lying across (*Kabete*), ×420. Fig. 29. A mature embryo sac with six antipodals resulting from division of the three antipodals; a two-nucleate aposporic embryo sac is encroaching upon this embryo sac; the nuclei of the aposporic embryo sac do not appear to be healthy. The mature embryo sac is probably haploid (*Rongai*), ×420. Fig. 30. A group of 5 embryo sacs in the same ovule. The embryo sac (1) to the right with the division figure is in all probability haploid. A two-nucleate embryo sac (2) almost perpendicular to the haploid embryo sac with one of its nuclei lying almost in the middle of

the haploid sac. A four-nucleate embryo sac; (3) with two of its nuclei near the division figure of the haploid embryo sac and the other two below the nucleus of the binucleate sac. The other two (4 and 5) are uninucleate embryo sacs one lying to left and the other below the group. The latter 4 appear to be aposporic (*Kabete*), ×420. Fig. 31. A four nucleate haploid embryo sac (*Kabete*), ×420. Fig. 32. Enlarged drawing of upper portion of Fig. 31 and showing 18 chromosomes, ×900. Fig. 33. Division figures in an aposporic embryo sac (*Kabete*), ×420. Fig. 34. Enlarged drawing of the upper division of Fig. 33 showing 36 chromosomes, ×900. Fig. 35. Two-embryo sacs with one embryo and well-developed endosperm in each. Multicellular, cœnocytic and vacuolate antipodals are seen even at this stage (*Kabete*), ×95. Fig. 36. *P. clandestinum* var. *Kabete*, $2n = 36$, ×900. Fig. 37. *P. clandestinum* var. *Rongai*, $2n = 36$, ×900.

sac and a uninucleate embryo sac lying across it also indicates a similar relationship, and further illustrates a rather later initiation of apospory. An extreme expression of this tendency of late origin of apospory is illustrated in Fig. 29. There are 2 embryo sacs here, one showing 6 antipodal nuclei formed by the first division of the antipodals, and the other, still in the binucleate condition, is found encroaching on the haploid mature embryo sac. The mature embryo sac is probably haploid in origin while the binucleate embryo sac appears to be aposporic. The shrivelled up nuclei of the binucleate embryo sac indicate that it is on its way to degeneration. Plate X, Fig. 6 is a photomicrograph of the same figure. Although it is usual for the aposporous initials to bring about the degeneration of the haploid embryo sac, especially if the former arise in the neighbourhood of the haploid embryo sac, it does not appear to be the case always. Several ovules show the presence of both aposporous and haploid embryo sacs developing simultaneously (Fig. 30). In this figure there are 5 embryo sacs and the curved embryo sac to the right containing the division figures is in all probability haploid while others are aposporic. The most important feature about this figure is that the aposporic initials in spite of their being (i) in close proximity to the sexual embryo sac; (ii) their aggressive growth; and (iii) their large numbers, have not been able to bring about the degeneration of the haploid embryo sac. The same situation is illustrated by Plate X, Fig. 4. The embryo sac to the right is 4-nucleate (only the upper 2 nuclei are visible in the photograph). The other embryo sac to the left is a mature one with an egg apparatus, 2 polars and 6 antipodals (only the 2 synergid nuclei, one polar and 1 of the antipodals are visible). As already stated, the difference in size between the nuclei of these 2 embryo sacs and their general appearance strongly suggest that the 4-nucleate embryo sac is haploid while the other is aposporic. It is quite possible that the aposporic embryo sac took its origin rather late and by a rapid series of divisions attained maturity earlier.

That both aposporic and haploid embryo sacs are formed in this species has been proved by cytological evidence. Fig. 31 shows a 4-nucleate embryo sac in which the upper 2 nuclei are in late prophase. In Fig. 32, which is

an enlarged drawing of the upper part of Fig. 31, 18 chromosomes, the reduced number can be counted proving the haploid nature of this embryo sac, while Fig. 33 shows an aposporic embryo sac with 2 division figures in metaphase. Figure 34 is an enlarged drawing of the upper part in which the unreduced number of 36 chromosomes could be counted. The cytological evidence supports the genetic findings described in a later section.

Fertilisation and Embryogeny

P. clandestinum var. *Rongai.*—Though the mature embryo sacs, whether aposporic or haploid, are well organised and healthy, examination at later stages of development disclosed the total absence of embryos. This is probably due to the absence of fertilisation. Such embryo sacs usually disintegrate and the ovule collapses.

Crossing Experiments

P. clandestinum var. *Rongai* (Plate X, Fig. 1) differs markedly from the *Kabete* variety (Plate X, Fig. 2) in two important features, *viz.*, (i) possession of non-exserted anthers and inability to form pollen and (ii) inability to set seed in the absence of pollen. Although in most situations under which it is found in cultivation, the *Rongai* variety sets no seed at all, Edwards (1937) states that it can set seed when growing in the neighbourhood of varieties producing pollen. This relation between the availability of pollen and the capacity to set seeds suggests that the plant is fertile on the female side and is capable of sexual reproduction. However, even though these varieties appear to be reproducing sexually and even though these differ from each other in such clear-cut characters as possession and non-possession of pollen and the exsertion and non-exsertion of anthers, no knowledge is available so far regarding the genetic basis of these characters.

For purposes of these investigations the *Kabete* variety which is capable of forming viable pollen and seed has been termed the male fertile variety, while the *Rongai* which lacks this ability to form pollen but has the potential capacity to set seed when it has access to pollen has been termed male sterile variety.

The two varieties show a remarkable resemblance to each other in displaying their stigmatic lobes by conspicuously exserting them. This feature of the *Rongai* variety facilitated the disposition of pollen on the exserted and plumose stigmatic lobes. Further, the question of emasculation which is such a difficult process in these grasses with florets hidden by the sheathing basal region of the leaf did not arise.

Hybridisation.—Before starting the hybridisation several hundred florets in the area of the *Rongai* variety were examined and were found to be without any seed. Pollination was conducted using the *Rongai* variety as the female plant. Heavy and repeated pollination was conducted to ensure success.

About a hundred florets that had been pollinated were examined for seed, and 20 seeds were harvested. Examination of a similar number of florets in the adjoining areas which served as the control disclosed a total absence of seed.

F_1 Generation.—Only 10 of the 20 seeds that were harvested were sown in flats in the greenhouse and seedlings raised. Seven out of the 10 seeds germinated and grew vigorously into plants under uniform conditions of the greenhouse. These were cloned and grown outside the greenhouse under natural conditions. Of these 7 plants, 2 closely resembled the maternal plants in not producing viable pollen and exserted stamens, while the other 5 produced good pollen and exserted stamens, and resembled the more fertile (*Kabete*) variety. This latter feature of the five plants suggested that they were hybrids and in addition indicated that the formation of exserted anthers and viable pollen is genetically dominant over the male sterility found in the *Rongai* variety. Furthermore, these plants resembled the parent plants in containing 36 somatic chromosomes, as disclosed by a count of the metaphase plates in the root tips.

The production in the F_1 generation of two types of plants, one type maternal, and the other resembling the pollen parent prompted the hypothesis that the *Rongai* variety is a facultative apomict reproducing both sexually and apomictically and that since pollen was essential for seed formation it is probably pseudogamous.

Evidence for the above hypothesis was sought directly by observations of the formation and development of the embryo sacs, for if the plants in question are apomictic this phenomenon should be disclosed in the method of embryo sac formation. Further, this study was necessary to learn the cytological mechanism involved in bringing about this apomixis. Actually, embryological investigations have disclosed the formation of both haploid and aposporous embryo sacs as already described. Formation of the hybrid plants in F_1 generation has been attributed to the fertilisation of the reduced eggs of the haploid embryo sacs by the reduced sperms resulting in the restoration of the parental chromosome number $2n = 36$, while the unreduced eggs of the aposporous embryosacs, under the stimulation of pollination

have developed into purely maternal plants with the same somatic chromosome number as the mother, $2n = 36$ (Fig. 37).

There is good correlation between cytological and genetical evidence in favour of the origin of maternal plants by apomixis and hybrid plants by the sexual method, *i.e.*, of the existence of facultative apomixis. But another possibility suggests itself. If one of the parental plants, *i.e.*, *Kabete* variety is heterozygous *Tt* for a dominant gene T responsible for the formation of viable pollen and exserted anthers, and the other parent is homozygous *tt* for its recessive allele *t* responsible for non-formation of pollen and non-exsertion of anthers, then also the 2 types of offspring obtained in the present investigations could be expected although in a 1 : 1 ratio. The number of offspring obtained in the present experiment is far too small to indicate whether a 1 : 1 or any other ratio was present. Since no other clear-cut morphological characters differentiate the two varieties, there is no way of telling whether the F_1 plants which resemble their maternal parents were actually hybrids or maternal parents produced by apomixis. Nevertheless the present cytological evidence based on direct observations of apomictic and haploid embryo sac formation strongly indicates that the plants in question are facultative apomicts.

DISCUSSION

Degeneration of the embryo sac.—Although it has been held by some students of apomixis (Rosenberg, 1907; Stebbins and Jenkins, 1939) that the physical destruction of either the embryo sac mother-cell or the developing megaspore is brought about by the aggressive growth of the aposporous initials, the simultaneous development of both sexual and aposporous embryo sacs in *Pennisetum clandestinum* indicates that physical destruction by the encroachment of the aposporic initials is not universal in all apomicts. Observations by Engelbert (1940), and Nielson (1946) on the simultaneous development of haploid and aposporic embryo sacs in *Poa* support such a view. Nevertheless all embryo sacs that start developing including the haploid ones may not reach maturity and some of them may succumb in the competition and degenerate. Whether it is the haploid ones that always succumb in this competition it is hard to say. Evidence for the view that the aposporic embryo sacs always overpower the haploid ones is not afforded by *Pennisetum clandestinum*. A reference to Fig. 29 and Plate X, Fig. 3 where the binucleate aposporic embryo sac is on its way to degeneration while the haploid embryo sac on account of the initial advantage gained by it has reached maturity in spite of the encroachment of the aposporous embryo sac strongly indicates that aposporous embryo sacs cannot always

bring about the physical destruction of haploid embryo sacs. Akerberg's (1943) observation in *Poa* that when a megaspore has reached a binucleate embryo sac stage it would easily be able to compete with the aposporous embryo sac initial and produce embryos supports the above assumption. Nielson's (1946) reference to Fig. 7 where a 4-nucleate haploid embryo sac is competing with a 2-nucleate aposporic embryo sac indicates that the haploid embryo sac is developing on account of the initial advantage gained by it. He favours the above view, *viz.*, that the aposporic embryo sac cannot always bring about the destruction of the haploid embryo sac in assuming that a simultaneous development of both sexual and aposporic embryo sacs to maturity in the same ovule is possible when " they are developing in an essentially synchronous manner".

Abnormalities in micro-sporogenesis.—The breakdown of meiosis leading to the various irregularities of micro-sporogenesis, *viz.*, resting pollen mother cells, breakdown of spindle mechanism and cytokinesis, etc., resulting in the formation of bent, twisted and tripolar spindles, formation of T-shaped and linear tetrads, etc., found in *Rongai* variety is not typical of apomicts although exhibited by this variety which is an apomict. Early stages of micro-sporogenesis in some pollen mother cells of *Rongai* variety have been found to be remarkably similar to those of *Kabete* variety. But in the majority of pollen mother cells the irregularities distinguishing the *Rongai* variety appear during different stages of pollen mother cell development. Since *Rongai* variety shows a breakdown of pollen mother cells while it forms normal embryo sacs on the female side, it is presumed to be a genetically determined male sterile form. Male sterility due to genetic causes has been reported by Eyster (1921), Beadle and McClintock (1928), Emerson, Beadle and Fraser (1935), and Fraser (1935) in maize; Lesley and Lesley (1939), and Rick (1944, 1945) in tomato. Male sterility due to a failure of cell division has been reported in *Datura* by Blakeslee, Avery *et al.* (1935). Beadle (1932) reporting a failure of cell division both in the first and second visisions in maize assumed that the conditions which produced the failure of cytokinesis in the first division continued to operate in the second division also. However, in this variety there are no instances of failure of cytokinesis in both the divisions.

The irregularities shown by this variety are believed to have a genetic cause. Such a belief is afforded by the results of the crossing experiments. The production of offspring resembling the male parent in the F_1 progeny of a cross between *Kabete* and *Rongai* indicates not only that these offsprings are hybrids but also the formation of normal pollen is dominant over male

sterility. These findings further suggest that male sterility in *Rongai* variety is caused by recessive mutations which arose independently of mutations which led to apomictic reproduction.

Pseudogamous apomixis.—In sexual forms seed formation proceeds only after double fertilisation. In apomicts there is a departure from this normal behaviour. In autonomous apomicts both the egg and endosperm develop without fertilisation. This is an extreme departure. In pseudogamous apomicts pollination is necessary but the embryo develops without fertilisation while the endosperm may develop with or without fertilisation. In cases where endosperm formation proceeds without fertilisation the mere germination of pollen tubes in the stylar tissue is supposed to stimulate endosperm formation. Such a view has been expressed by Haberlandt (1922 *c*) cited by Gustafsson (1947), Boos (1924), Haflinger (1943), and Engelbert (1941). However, a large number of workers now hold that apomicts usually require fertilisation of the secondary nucleus for the initiation of endosperm development. Noack (1939) in *Hypericum*, Gentscheff and Gustafsson (1940) in *Potentilla*, Akerberg (1943) in *Poa* have shown that triple fusion is absolutely necessary for the development of the endosperm.

In *Rongai* variety the inability to form seed in the absence of pollination and the relative ability to form seed which give rise to normal plants after pollination establishes the fact that *Rongai* is pseudogamous. Whether this pseudogamy conforms to the former type requiring only pollination and no fertilisation, or whether it requires fertilisation of the secondary nucleus for the initiation of endosperm development is not clear. Focke (1881), cited by Brink and Cooper (1947) who introduced the term pseudogamy supposed it to involve the formation of an embryo without fertilisation but with pollination, and such a view is shared by Stebbins and Jenkins (1939) who define pseudogamy as the stimulation of an egg by the male gamete but without complete fertilisation. In *Pennisetum clandestinum* although it is not clear whether it is the mere presence of the sperm nuclei in the carpellary tissue, or the formation of the endosperm after fertilisation that stimulates the formation of the embryo, the formation of hybrid plants which require fertilisation of the egg suggests that it is the latter, *viz.*, stimulation of secondary nucleus by fertilization.

SUMMARY

P. clandestinum consists of two varieties, *viz.*, *Kabete* and *Rongai*. The *Rongai* variety showed abnormalities of micro-sporogenesis resulting in poor pollen and non-exserted stamens and did not form seed when isolated although it showed fertility on the female side by forming seed when it had

access to pollen. The *Kabete* variety had normal pollen and exserted anthers and formed good seed. Seeds obtained by hybridising the two varieties using the *Rongai* as the female parent, gave rise to maternal plants and hybrids with viable pollen and exserted anthers, showing not only the recessiveness of the male sterility in *Rongai*, but also indicating facultative apomixis. This indication was confirmed by cytological evidence based on a chromosomal count showing the formation of haploid and aposporic embryo sacs. Embryo formation is normal in *Kabete* and is preceded by endosperm formation. Polyembryony occurs.

Acknowledgments

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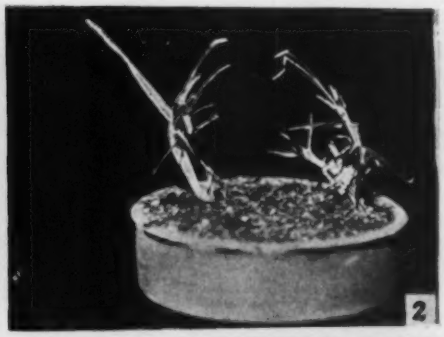
EXPLANATION TO PLATE

Figs. 1–6. Fig. 1. *Pennisetum clandestinum* var. *Rongai*, showing the bifid stigma. Fig. 2. *P. clandestinum* var. *Kabete*, showing the exserted anthers. Fig. 3. *P. clandestinum* var. *Kabete*, first anaphase showing 18 bivalents. Fig. 4. *P. clandestinum* var. *Kabete*, portion of the embryo sac to the right with 2 nuclei at the micropylar end. This embryo sac is probably haploid. E. sac to the left with two hooked synergids and one of the polars is probably aposporic in origin as is indicated by the relative size of the nuclei. Fig. 5. *P. clandestinum* var. *Kabete*, embryo showing two-celled embryo with well-developed endosperm and multicellular, vacuolate and persistent antipodals. Fig. 6. *P. clandistinum* var. *Rongai*, showing two embryo sacs; to the left is aposporic; the mature embryo sac to the right may be haploid.

WAVE

1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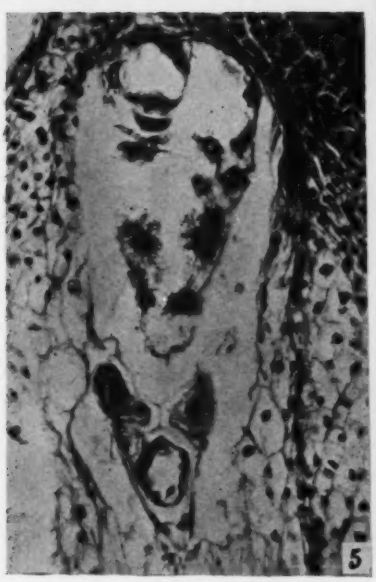
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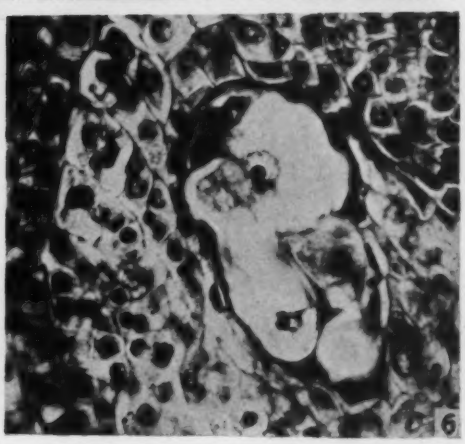
6

STUDIES ON ACETOBACTER

I. Isolation and Characterization of the Species

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The importance of acidity development in the alcoholic liquors has been realized from the very early times but it is only during this century that the exact role of acetic acid bacteria in the acetification process is being understood. The recent recrudescence of interest in these bacteria is another strong pointer to their increasing importance and many attempts have been made to utilize them even in industries other than in the manufacture of vinegar (Prescott and Dunn, 1949). But in our country even the quality of vinegar produced and marketed lack many of the attributes associated with a good product and leave much room for improvement. Furthermore the raw materials suitable for vinegar manufacture are available in abundance and invite exploitation for the economic development of our country. As a first step towards this, it was considered worthwhile to examine varieties of materials for the presence of these bacteria and indicate the sources which would help bring about the desirable type of fermentation for the industrial production of vinegar. In this paper are presented the details of the methods employed for isolation, characterization and identification of Acetobacter, a genus in which may be found species neither well defined nor fully described. It would also be clear from the comparative descriptions presented that some of the species placed by Bergey, *et al.* (1948) in the appendix to the genus Acetobacter (p. 185–89) deserve as much recognition as the better-characterized species mentioned under the key to the genus. In this paper are also presented the characteristics of some isolates which fit in with the descriptions given for Acetobacter but which differ from it by forming gas in the carbohydrate media.

Materials and Methods

The thirty odd species mentioned in this paper were isolated by us from such diverse sources as grapes (*Vitis vinifera* L.), mangoes (*Mangifera*

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indica L.), pine-apples (*Ananas sativa* L.), sapotas (*Achras sapota* L.), oranges (*Citrus aurantium* L.), plums (*Prunus domestica* L.), strawberries (*Fragaria indica* Andr.), bananas (*Musa sapientium* L.), beets (*Beta vulgaris* L.), tomatoes (*Lycopersicum esculentum* Mill), raisins (*Vitis vinifera* L.), dates (*Phœnix dactylifera* L.), jaggery (unclarified sugar), alcoholic liquors (neera, drakshasava and beer), soils, human and cow excreta and as aerial contaminants. Where extraction of the juice was possible from the cleansed material, the extracted juice served as the inoculum; where this was not possible, a portion of the material was directly introduced into the medium utilized for isolation purposes.

Though various complex media have been recommended for the isolation of acetic bacteria, there have not been many attempts directed at culturing them exclusively on simple, chemically defined media such as the one designed by Beijerinck (1898). This medium stands out as the simplest and offers a satisfactory approach to the isolation of the acetic acid bacteria by resort to the enrichment culture methodology. The presence of alcohol in this medium (see composition below), besides inhibiting the growth of several bacteria, serves as a 'selective' carbon and energy source for the acetic acid bacteria. Furthermore, the medium does not appear to be favourable for the growth of several other bacteria with the result that they get easily eliminated during the enrichments. The composition of this medium is as follows:

Tap water	100 ml.
$(NH_4)_2HPO_4$	0·05 g.
KCl	0·01 g.
Ethyl alcohol† ..	3 ml.
pH adjusted to ..	6·5

This medium dispensed in 50 ml. aliquots in 250 ml. Erlenmeyer flasks was inoculated and incubated at room temperature (27 to 29° C.) until growth appeared (room temperature was observed to be better than incubation at 37° C.). The growth was examined microscopically and passed through two successive transfers in the same medium, the transfer being effected each time after a critical microscopical examination. Two transfers usually resulted in the elimination of the extraneous flora so much so that the third enrichment on streaking on the same medium solidified with agar (and containing a few drops of Andrade's indicator) was invariably found to give rise to colonies mostly made up of the acetic acid bacteria which could be

† Alcohol was incorporated after sterilization of the salt solution by autoclaving.

detected by their pink colony formation. The colonies fished out and examined were repurified and tested for their power of acetifying alcohol by titration methods using in these tests various concentration of alcohol ranging from 2 to 8 per cent.

In some instances, the contents from the third enrichments failed to give any growth on the corresponding solidified medium or gave only poor growth after a long incubation period. In these cases, apple juice agar of the following composition was made use of for isolation purposes.

Apple Juice Agar

Tap water	.. ..	100 ml.
$(NH_4)_2HPO_4$	.. ..	0·2 g.
Apple juice	.. ..	2% (v/v)
Agar	.. ..	3 g.

The pure cultures thus obtained were tested for the production of acid from alcohol. Though Beijerinck's solution was found unsuitable for this purpose, the substitution of 0·5 per cent. peptone in some instances and 0·2 per cent. yeast extract in others for the ammonium phosphate, yielded satisfactory results. The various isolates were then carefully examined for their morphological characteristics such as shape, size, motility, capsule formation, swollen cell and thread formation as well as for various cultural and physiological peculiarities such as pigment production, giant colony formation, nature of growth on solid and liquid media, gelatine liquefaction, changes in litmus milk, growth in Hoyer's medium, behaviour in presence of ethyl alcohol, oxidation of acetic acid to carbon dioxide, production of acid and gas from carbohydrates and alcohols, production of dihydroxy acetone from glycerol and the formation of sorbose from sorbitol. Though a large number of media and their modification were utilised for each test, only those which yielded consistent and reliable results were adopted for interpretation. It may be mentioned in this connection that in general, proteose peptone (Difco) was found to be a better source of nitrogen than other peptones, and yeast extracts as a rule hastened growth and development of results.

Results and Discussion

The identification of acetic acid bacteria has been rendered difficult because the genus has been encumbered by a large number of poorly described species. Of the thirty odd species known and described in the literature, only a few are well known and the rest may be regarded as unrecognizable. As the purpose of this paper will be better served by a detailed

description of the less-known species with only indication of differences observed from the well-known ones, species differentiations are not presented here beyond a reference to variability of behaviour, a factor to be reckoned with in all species. For instance, some acetic acid bacteria acquire the power to produce acid from a sugar or alcohol which it normally cannot utilise whereas others may lose their ability to form acid from the same substrates. Therefore, neither fresh criteria to differentiate species nor the creation of new species has been attempted. By a critical study and utilisation of the criteria previously employed, a comparative study of new isolates is presented, which we hope, will help remove some of the existing confusion and render the classification of the genus *Acetobacter* more clear. The thirty species described in this paper have been grouped arbitrarily on the basis of cultural and gross physiological characteristics.

Group I. Oxidise ethyl alcohol to acetic acid and further to carbon dioxide; utilise ammonium salts as the sole source of nitrogen.

Eight isolates from our collection fall in this group and in conformity with the opinion of Hoyer (1898) and Beijerinck (1898) we recognize these isolates as *A. aceti* without referring them to any particular variety (strain) of the parent species. We however wish to point out here that a typical *A. aceti* is said to produce acid from glucose, ethyl alcohol, propyl alcohol and glycol and no acid from methyl, isopropyl and butyl alcohols, glycerol, fructose, lactose, maltose, or sucrose to mention only some common laboratory tests. However, some of our strains fermented lactose, fructose, maltose and sucrose as well as butyl and isobutyl alcohols. One strain even fermented glycerol. None of the strains however produced acid from methyl or propyl alcohols. One strain also showed such a strong tendency to ascend the sides of the glass containers that we are constrained to regard it as *Acetobacter ascendens*, a species placed in the appendix to the genus in *Bergey's Manual*. We give below data which would supplement the description in *Bergey's Manual* and make identification of this species less difficult.

Morphology.—Cylindrical to ellipsoidal rods (average size $1\,\mu/0{\cdot}7\,\mu$) occurring singly, in pairs and in irregular clumps. Thread and swollen cell formation (at 40° C.) absent, capsulated, non-motile, non-sporulating; Gram negative and do not give the cellulose reaction with iodine solution.

Growth on Beijerinck's solidified medium.—Round, smooth, glistening, convex, entire colonies less than 1 mm. in diameter.

Growth on Apple juice agar.—Round, smooth, glistening, convex colonies with an undulating margin, opaque in the centre and transparent on the borders, size 1–2 mm.

Giant colonies on Beijerinck's semi-solid agar.—Smooth, opaque, white glistening colonies, 2·1 cm. in diameter.

Growth on yeast extract-glucose—$CaCO_3$ agar.—Smooth, flat, glistening transparent colonies.

Growth in yeast extract broth.—White thin film with a strong tendency to ascend the glass wall.

Growth in yeast extract—glucose broth.—Same as above with added turbidity of the liquid medium.

Physiological characteristics.—Catalase positive; gelatine not liquefied; produce acid and soft curd in litmus milk; litmus reduced; grows luxuriantly in Hoyer's mineral solution. Tolerates alcohol upto 10 per cent. (v/v) and produces acid after a week. Forms acid from glucose, lactose, sucrose, ethyl alcohol, propyl alcohol, butyl alcohol and isobutyl alcohol; does not ferment maltose, fructose, methyl alcohol, isopropyl alcohol, amyl alcohol or glycerol; does not form dihydroxy acetone from glycerol medium.

Source.—Soil.

Group 2. *Oxidised ethyl alcohol to acetic acid and further to CO_2; do not utilise ammonium salts as the sole source of nitrogen.*

Eight other isolates could be placed in this group which include species such as *A. xylinum*, *A. acetigenum* and *A. xylinoides* characterised by their ability to produce a cellulosic pellicle. Only one isolate in our stock could be demonstrated to form a cellulosic pellicle and this could be referred to as a variant of *A. xylinum* inasmuch as it failed to form dihydroxy acetone and formed acid from isopropyl and isobutyl alcohols. Another species could be labelled as *A. pasteurianum* despite its ability to produce slight acid in lactose, butyl alcohol and glycerol. A third strain has been referred to as *A. rancens* despite the fact that it differed from a typical strain by its failure to produce acid from butyl alcohol. Another strain has been referred to by us as *A. viniaceti* inasmuch as it agreed in general with this species as described in Bergey's appendix to the genus. However, some additional data on the species compiled by us are briefly presented below:—

The organism is encapsulated and non-motile.

On Beijerinck's solidified medium.—Smooth, white glistening colonies: similar but larger colonies are formed on apple juice agar. No giant colony formation on semi-solid apple juice agar.

On yeast extract-glucose—$CaCO_3$ agar.—Pale yellow, abundant, glistening moist, opaque growth.

Catalase present—Gelatine not liquefied. Acid and soft curd in milk; litmus reduced. Very slight growth in Hoyer's mineral solution after 2 weeks. Tolerate 8 per cent. (v/v) alcohol in which acid is formed after 10 days. Oxidation of alcohol and acid slow but complete. Slight acid from lactose. Produces dihydroxy acetone from glycerol medium.

Source.—Pine-apple.

Two other strains isolated one each from human intestine and jaggery are referable to as *A. plicatum* even though some minor differences are indicated in their physiological behaviour. Both were Gram negative, non-motile, capsulated, occurring singly, in pairs or short chains; the one from human intestine showed few swollen cells. Both give smooth, moist, glistening, transparent to transluscent, cream coloured, round, entire colonies on apple juice agar, but do not form giant colonies on the semi-solid medium of the same base. In yeast extract glucose medium viscous growth on top with a tendency to ascend the wall. In yeast extract ethyl alcohol medium, sticky growth at the bottom and on yeast extract-glucose-$CaCO_3$ agar cream coloured moist, watery, transluscent, raised, butyrous growth. Catalase present; gelatine not liquefied; litmus reduced and acid formed in milk; no growth in Hoyer's mineral solution; tolerates 5 per cent. alcohol in which growth appears after 24–36 hours and acid formation after 2 weeks. Oxidation of alcohol and subsequently of acid complete. Acid from glucose, maltose, fructose, ethyl alcohol; human strain also from sucrose and isobutyl alcohol; jaggery strain from propyl alcohol; no acid from methyl, isopropyl, butyl, amyl alcohol or glycerol.

The remaining two isolates presented similarities to *A. turbidans* by forming transparent to transluscent colonies with oil-like consistency on apple juice agar and wort agar: Giant colony formation was absent; on yeast extract glucose-$CaCO_3$, white, moist, watery, opaque, butyrous growth. In yeast extract medium, growth along the sides of the tubes with a scanty deposit. In the same medium with glucose, ring, and liquid very turbid. In ethyl alcohol yeast extract medium the growth was good with a yellowish powdery deposit; catalase positive; no liquefaction of gelatine; reduction of litmus; acid and soft curd in milk. No growth in Hoyer's medium; slight growth in 8 per cent. alcohol, acid being detectable after 8 days; oxidation of alcohol to acid and to CO_2 complete; glucose, ethyl alcohol, isobutyl alcohol and glycerol only fermented to form acid. No acid in lactose, maltose, sucrose, fructose, propyl and isopyropyl alcohol, butanol, methanol and amyl alcohol. The ability to form tense density in beer only remains to be checked.

Source.—Soil.

Group 3. *Oxidise ethyl alcohol to acetic acid only and not completely to* CO_2; *show pigmented and ropy growth.*

Only three isolates could be placed in this group. One of these isolated from soil agreed well with *A. melanogenum*. It formed a deep brown pigment on glucose yeast extract—$CaCO_3$ agar after 2 weeks. The chromogenic property was enhanced in litmus milk. The culture formed l-sorbose from l-sorbitol and reduced Fehling's solution completely; formed dihydroxy acetone from glycerol: acid formed in glucose, lactose, sucrose, ethyl, propyl and butyl alcohols, but not in maltose, fructose, methyl, isopropyl, isobutyl or amyl alcohols. Only slight acid in glycerol.

Another strain could be tentatively identified as *Acetobacter viscosum*, again placed by Bergey *et al.*, in the appendix. This was described by Shinwell (1948) and characterised by the formation of ropiness or rendering the liquid media viscous. It produced also very slimy growth on solid media; thick white growth on Beijerinck's solid medium; clear ropiness in Hoyer's mineral solution; formation in 7 days of 2 cm. smooth moist and transparent colony on semi-solid medium and viscosity in liquid media containing ammonium cations as the sole nitrogen source are some other peculiarities of this species.

The third isolate belonging to this group agreed with some characteristics of *A. aceti* on the one hand and *A. roseum* on the other. Because of its distinct rose coloured colony formation on all media employed, it should be characterised as *A. roseum*. One strain utilized ammonium salts as sole source of nitrogen and formed dihydroxy acetone but we do not know if *A. roseum* actually does so. This was isolated from mango whereas the strain described in Bergey has been referred to have been isolated from fermenting mash of dried persimmons and figs and dates.

Group 4. *Oxidise ethyl alcohol to acetic acid but not to* CO_2; *oxidise a large number of carbohydrates.*

Of the five isolates in our collection placed in this group, two isolates from plum and banana respectively could be identified as *A. oxydans* whereas the rest secured from soil, *neera* (unfermented toddy) and berry (*Zizyphus rotundifolia*) were referred to as strains of *A. industrium*. It is necessary to point out here that *A. oxydans* has been given the primary status of a recognized species in *Bergey's Manual* whereas *A. industrium* is given only a secondary status in being placed among the 17 species mentioned in the appendix. Inasmuch as both are not well described in the *Bergey's Manual*, their characteristics are presented below.

Morphology.—The morphological form varied from the coccoidal form to rods nearly 2 μ in length, the cells being arranged singly, in pairs, or in short chains. Branched and swollen cell formation were observed with the 2 strains placed as *A. oxydans* but not with the other strains. All 5 strains were capsulated, non-sporulating, Gram negative and as a rule non-motile, though one strain each of *A. oxydans* (from banana) and *A. industrium* (from berry) in our collection exhibited slight motility. Though *Bergey's Manual* describes *A. oxydans* as typically motile, this has been shown to be a variable character by Jorgensen (1939) and Shinwell (1948).

Growth on Beijerinck's solidified medium.—All 5 strains showed poor growth on this medium, colonies being pin point and barely visible.

Growth on apple juice agar.—Round, smooth, glistening, opaque colonies with an entire to undulating margin. About 1–2 mm. in diameter. Four of the 5 strains showed a cream coloured to yellowish pigment, the strain of *A. oxydans* from banana being the only one showing no pigmentation.

Giant colonies on Beijerinck's semi-solid agar.—Absent.

Growth in yeast extract broth.—The 2 strains placed as *A. oxydans* gave poor particulate growth while the three other strains gave a good uniform growth. In addition the strain isolated from berry produced a thin ascending film.

Growth in yeast extract—glucose broth.—Same as above with added turbidity.

Growth in yeast extract—ethyl alcohol broth.—Floccular growth and ring which is yellowish in the case of the *A. industrium* strains. Some strains also show a powdery deposit.

Growth in yeast extract—glucose—$CaCO_3$ agar.—Smooth, opaque, slightly raised glistening colonies ranging in colour from pale yellow to pink and orange.

Physiological characteristics.—Catalase positive, gelatine not liquefied; litmus reduced with the production of acid by all strains except *A. industrium* from soil which shows alkaline peptonisation and *A. oxydans* from banana which shows only reduction and peptonisation. No growth in Hoyer's mineral solution. Ethyl alcohol tolerated upto a concentration of 5–7 per cent. (v/v), acid being produced in it after long incubation (5–20 days). Oxidation of ethyl alcohol incomplete. Glucose, maltose, sucrose, fructose, galactose, dextrin, arabinose, xylose and ethyl alcohol fermented with acid only. Lactose not fermented by one strain of *A. oxydans* (from banana) while others ferment it. One strain each of *A. oxydans* (from plum) and

A. industrium (from berry) produce acid from propyl alcohol while isopropyl alcohol is not fermented by any strain. *A. oxydans* from plum also produces acid from butyl alcohol but not isobutyl alcohol while the strain from banana produces slight acid from isobutyl alcohol and none from butyl alcohol. All 3 strains of *A. industrium* fail to produce acid from butyl alcohol but the strain from *neera* and berry do so from isobutyl alcohol. Dihydroxy acetone was produced from glycerol by the 2 strains placed as variants of *A. oxydans* on this account. Traces of dihydroxy acetone were also produced by the strains of *A. industrium* isolated from *neera* and berry.

Group 5. Produce acid and gas from carbohydrates.

In this group showing the unusual property of producing gas from some carbohydrates were placed six strains from one collection isolated from such diverse sources as spoilt beer, soil, orange, apple and *drakshasa* (grape liquor). Inasmuch as no strain of *Acetobacter* is known to form acid and gas from carbohydrates or alcohols, our strains could not be placed in any of the known species and are described in detail here.

Morphology.—Cylindrical to ellipsoidal rods (average size 1–2 μ/ 0·5–0·8 μ or 0·5–0·6/0·7–0·9 μ) occurring singly and in pairs. Swollen cell formation shown by 4 strains only (from 2 soils, orange and apple) Gram negative, non-motile and non-sporulating.

Growth on Beijerinck's solidified medium.—Round, smooth, moist, glistening translucent colonies with an entire margin, about 1–2 mm. in diameter.

Growth on apple juice agar.—Luxuriant growth. Colonies within 20 hours round, smooth, moist glistening and slimy with an entire margin and 1–3 mm. in size.

Giant colonies on semi-solid apple juice agar.—Smooth, slightly raised glistening, semi-transparent colonies varying in size from 0·5–3·2 cm. The strains from Nasik soil and *Drakshasa* produced more opaque colonies.

Growth in yeast extract broth.—The strain from spoilt beer showed only a heavy floccular deposit at the bottom while all other strains in addition showed growth with a tendency to ascend the glass wall.

Growth in yeast extract—glucose broth.—As above, but heavier growth and brownish coloured sediment.

Growth in yeast extract—ethyl alcohol broth.—White film or ring with a tendency to ascend glass wall. Uniform turbidity and floccular deposit.

Growth on yeast extract—glucose—$CaCO_3$ agar.—Abundant to moderate smooth moist, growth with gas bubbles at the butt of the slant.

Physiological characteristics.—Catalase positive; gelatine not liquefied; litmus reduced with acid and soft curd; good growth in Hoyer's mineral solution; alcohol tolerance ranged from 6–10%, acid being produced after a time. Oxidation of ethyl alcohol, incomplete. Acid and gas produced from glucose, lactose, maltose, sucrose, fructose, mannitol and glycerol; and acid only from ethyl alcohol. The strains from Nasik and Bombay soils produced no acid from propyl, isopropyl, butyl and isobutyl alcohols. Strains from spoiled beer, orange, apple and *drakshasa* produced acid from butyl and isobutyl alcohols. In addition, the isolates from orange and *drakshasa* also produced acid from propyl and isopropyl alcohols. A trace of dihydroxy acetone was produced by isolates from spoiled beer and Bombay soil whilst all other isolates did not do so.

The production of acetic acid by these 6 strains was confirmed by steam distilling the fermented liquid, neutralising the distillate with sodium hydroxide, evaporating to dryness, dissolving the dried mass in a small quantity of water and adding neutral $FeCl_3$ to it. The formation of a brown precipitate of basic ferric acetate on boiling confirmed the presence of acetic acid. Inasmuch as bacteria other than *Acetobacter*, such as the heterofermentative lactic acid bacteria, Coli-aerogenes group of bacteria and certain anaerobic sporeformers are also known to produce acetic acid, additional tests such as the methyl red and Voges-Proskauer tests were also carried out and found to be negative. Gas production was maximum in 1 week (0·8–2 ml.) from 10 ml. of liquid medium and the nature of the medium and age of culture had some effect on the amount of gas produced. The formation of CO_2 from glucose under certain conditions by *A. oxydans* (Butler, 1936, 1938) and from arabinose and xylose by *A. xylinum* and *A. sorbose* (Fred *et al.*, 1923) has been reported in the literature but the fermentation of a large number of sugars and alcohols with the consistent production of gas has not been so far attributed to *Acetobacter* species, and deserve further investigations.

SUMMARY

A study of 30 strains of *Acetobacter* species from diverse sources such as air, soil, various fruits, fermented liquors and animal excreta was attempted. By a detailed characterization of the isolates, they could be divided into 5 groups and identified as species of *A. aceti*, *A. xylinum*, *A. pasteurianum*, *A. melanogenum*, *A. industrium*, *A. oxydans*, *A. aceti viscosum*, *A. aceti roseum*, *A. rancens* and their variants. Detailed descriptions of the less known species

are presented to supplement the information available in appendix to the genus given in the *Bergey's Manual*. The description of a group of 6 strains which possess the unusual property of fermenting several carbohydrates with the production of acid and gas—a property not thus far ascribed to *Acetobacter* species—is also given.

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NATURAL TETRAPLOIDY IN *CICER ARIETINUM* L.

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Introduction

Argikar[1] reported a mutant in gram (*Cicer arietinum* L.) which did not cross with normal gram and consequently it was found worthwhile to investigate it cytologically. This mutant was noticed in a crop of 'Chafa' variety of gram grown at Dharwar in 1944. On examination it was observed that the mutant was a tetraploid with a somatic chromosome number of 32. Ramanujam and Joshi[5] have described in detail colchicine-induced polyploidy in gram but naturally occurring polyploid such as the one under consideration has not been reported so far. In the present article cytological observations made on this mutant material are reported. Iyengar[3] has made tentative inferences regarding the general morphological categories of the somatic complement of *Cicer arietinum*. In the present contribution a detailed and critical analysis of morphology of the individual chromosomes in the somatic complements of the diploid as well as the tetraploid types has been given for the first time.

Material and Method

Seeds of both the mutant and the normal types of gram were germinated in 6-inch pots containing 50 : 50 soil and sand. After 3 to 4 days when the roots had become 2 to 3 cm. long they were severed from the seedlings and fixed in Craf's solution (2 A : 1 B) at 11 A.M. for 24 hours, dehydrated and embedded in the usual manner. Each tip thus represented a separate individual plant. Sections of 12 μ were cut and stained in crystal violet after mordanting for 1 hour in 1% chromic acid. For meiotic studies smears were made between 8 and 11 A.M. using Craf's solution (2 A : 1 B) and stained in crystal violet after mordanting in 1% chromic acid. Acetocarmine preparations were used in examining tetrad abnormalities. Pollen grains were germinated on slides previously smeared with a solution of 1% agar mixed with varying percentages of cane sugar and the preparations were made permanent by the method described by Maheswari and Wulff[4] using gentian violet for staining. All figures (excepting Figs. 14 to 18) were drawn at table level with the aid of a prism camera lucida using $\times 95$ oil immersion objective,

an aplanatic condenser N. A. 1·25 and a compensating ×20 ocular, giving a total magnification of approximately ×3000. Figures 14 to 18 were drawn under different magnifications as shown in the legends accompanying the figures.

Experimental Results

In all 20 root tips, each belonging to a different seedling were examined for somatic number. Out of these, 16 root tips showed 32 chromosomes (Fig. 2). The remaining 4 tips showed the presence of 33 chromosomes (Fig. 3). Observations regarding the morphology of the chromosomes are given in Table I below.

Table I

Karyotypes of diploid and natural autotetraploid gram

Class	Position of construction		Length of component segments in μ	Total length in μ	Mean diameter in μ	Total No. of chromosomes.	Total length of complement in μ	Mean length of chromosome in μ
	Primary	Secondary						
	Diploid Chafa gram							
1	Median		0·50+0·50	1·00	0·33	2	2·00	
2	,,		0·72+0·72	1·44	0·33	4	5·76	
3	,,		1·00+1·00	2·00	0·33	2	4·00	
4	Sub-median		0·67+0·83	1·50	0·33	2	3·00	
5	,,		0·67+1·00	1·67	0·33	2	3·34	
6	,,		0·83+1·08	1·91	0·33	2	3·82	
7	,,	Satellited	1·08+1·20 +0·42	2·70	0·33	2	5·40	
						16	27·32	1·71
	Autotetraploid gram							
1	Median		0·50+0·50	1·00	0·33	4	4·00	
2	,,		0·75+0·75	1·50	0·33	8	12·00	
3	,,		1·00+1·00	2·00	0·33	4	8·00	
4	Sub-median		0·67+0·83	1·50	0·33	4	6·00	
5	,,		0·67+1·00	1·67	0·33	4	6·68	
6	,,		0·83+1·20	2·03	0·33	4	8·12	
7	,,	Satellited	1·16+1·21 +0·4	2·77	0·33	4	11·08	
						32	55·88	1·74

FIGS. 1–12. Camera lucida drawings. Fig. 1. Somatic metaphase of 'Chafa' gram (Diploid) showing $2n = 16$ chromosomes. Magnification, ×3000. Fig. 2. Somatic metaphase of autotetraploid gram showing $2n = 32$ chromosomes. Satellited chromosomes are the largest and are marked S in the figure. Magnification, ×3000. Fig. 3. Somatic metaphase of autotetraploid gram showing 33 chromosomes. Satellited chromosomes are marked S. Magnification, ×3000. Fig. 4. Variations observed in satellited chromosomes in the somatic cells of the autotetraploid gram. Magnification, ×3000. Figs. 5 to 9. P.M.Cs showing different valencies of chromosomes and configurations at metaphase I of the autotetraploid gram. Magnification, ×3000. Fig. 10. P.M.C. showing incipient anaphase I separation in the autotetraploid gram. Quadrivalents separating two by two Magnification, ×3000. Fig. 11. Lagging chromosomes in the anaphase II of the autotetraploid gram. Magnification ×3000. Fig. 12. 15–17 distribution of chromosomes at meiotic metaphase II in the autotetraploid gram. Magnification, ×3000.

It will be seen from the above table that the tetraploid gram possesses a closely similar chromosome complement as compared to the diploid (Figs. 1, 2 and 13). Diploid gram showed one pair of satellited chromosome and the tetraploid gram showed two pairs as expected (Figs. 1 and 2). The

author did not notice, however, the second pair of satellited chromosome in diploid gram as observed by Iyengar.[3] The presence of such pairs was also not noticed in the tetraploid complement. Decrease in size of chromosomes with increase in chromosome number was not observed in the tetraploid material examined (Figs. 1 and 2). Satellited chromosome pairs in the tetraploid gram showed variations in the length of the constriction in the satellited portion (Fig. 4). This may be due to variation in the degree of spiralization of the chromonemata in that region.

Examination of 20 P.M.Cs of the mutant at metaphase I, polar and side views showed the following distribution of chromosomes.

TABLE II

Distribution of chromosomes at metaphase I ($2n = 32$)

Number of cells	Chromosome valency				Total number of chromosomes
	Uni	Bi	Tri	Quadri	
7	..	..	..	8	32
4	..	4	..	6	32
3	1	2	1	6	32
3	..	2	..	7	32
1	1	..	1	7	32
1	4	2	..	6	32
1	..	10	..	3	32

The maximum number of quadrivalents appeared to be eight (Fig. 8). Figures 5 to 9 show different valencies and configurations of quadrivalents. In about 50% of the P.M.Cs anaphase I was regular and in majority of such cases quadrivalents were separated two by two (Fig. 10). In about 45% of the cells unequal distribution of the type 15 + 17, was observed at Metaphase II polar view, Fig. 12. In anaphase II unequal distribution was observed in rare cases and in such cases 2 to 3 chromosomes were found to lag at the equator of the second meiotic division (Fig. 11). One hundred and ten P.M.Cs examined to find out distribution of microspores *plus* micronuclei gave the following results:—

TABLE III

Distribution of microspores plus *micronuclei at the end of meiosis in autotetraploid gram*

No. of microspores *plus* micronuclei	3	4	5	6	7	8	Total P.M.Cs	Normal P.M.Cs %
No. of P.M.Cs	5	62	34	6	2	1	110	56

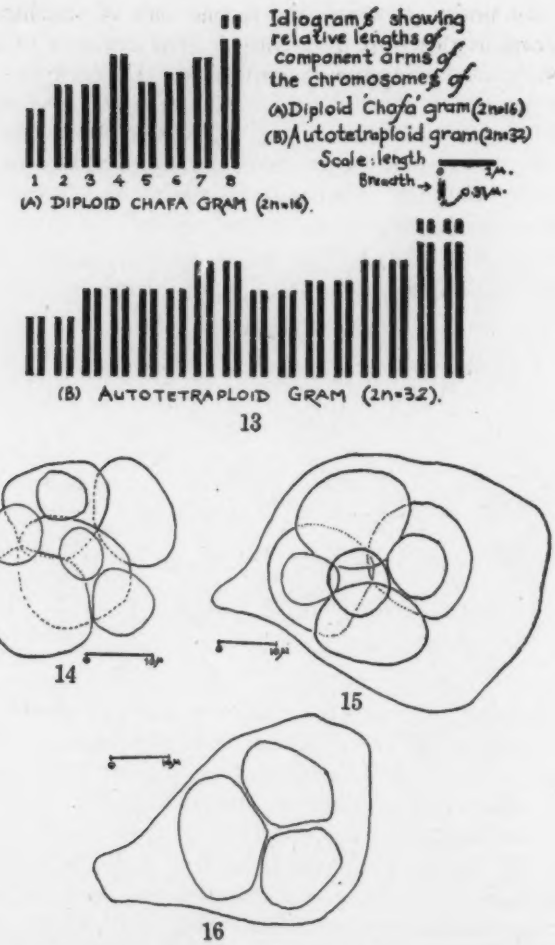

Fig. 13. Idiogram showing relative lengths of the component arms of the chromosomes of (*a*) Chafa gram (diploid), and (*b*) autotetraploid gram. Fig. 14. Abnormal tetrads showing 8 bodies in the P.M.C. of autotetraploid gram. Magnification, ×1500. Figs. 15–16. Aceto-armine preparations showing number of bodies contained in abnormal P.M.Cs. in the autotetra-gram. Magnification, ×1300.

56% of the P.M.Cs contained normal tetrads with tetrahedral arrange-ment. Figures 14, 15 and 16 show abnormal P.M.Cs containing 3 to 8 microspores *plus* micronuclei each having a separate wall. There are inter-grades normal, big and small in the size of microspores. All these have been collectively called as microspores *plus* micronuclei, abnormally small

ones being classed as micronuclei. These micronuclei are each enclosed in its cell wall.

Though appreciable variation in pollen grain size in mutant gram was observed (Fig. 17) about 60% of the pollen grains germinated when tested in sugar-agar solution. Diploid gram possessed regular but comparatively small sized pollen grains (Fig. 18) and about 85% of them germinated when tested in sugar-agar solution. Maximum germination was obtained on 12% sugar solution in the case of tetraploid gram and on 7½% solution in the case of diploid gram. This differential behaviour shows that increase in ploidy has affected the osmotic concentration, a physiological character.

Discussion

As generalised by Avdulov[2] from his observations on large number of polyploid species, in most cases there is usually decrease in chromosome size with increase in ploidy. This does not seem to hold good in the present case. The synthetic autotetraploid gram obtained by Ramanujam and Joshi[5] is being subjected to continuous selection for high fertility and individual plant yield at the I.A.R.I., New Delhi. This increase in fertility is presumably due to progressive increase in the coefficient of bivalency from generation to generation. In the present type, studied here, coefficient of multivalency is fairly high. It may have to be subjected to continuous selection for some generations before a fairly constant type with maximum divalent associations and having desirable agronomic attributes can be isolated. Occurrence of extra chromosomes is common in the progenies of autotetraploids where all possible variations in the number of chromosomes are expected with a modal frequency value of the tetraploid number as for example 32 in the present study. It may, therefore, be possible to come across root tips of higher chromosome numbers say 34 to 36 and more if larger number of root tips are examined though of course the chances of occurrence of higher chromosome number will be less. In such cases, the extra chromosomes may resemble one or more chromosomes of the diploid. The fact that the mutant does not cross with a diploid is due to its tetraploid constitution. The fact that the maximum germination was obtained in 12% sugar solution in the case of tetraploid gram and 7½% in the case of diploid gram indicates that the tetraploid has higher osmotic pressure indicative of higher concentration of water-soluble cell contents.

Summary

Cytological observations on the naturally occurring mutant gram have been described. The mutant was found to be an autotetraploid with $2n=32$.

Some seedlings with one extra chromosome were observed. Occurrence of such extra chromosomes is common in the progenies of autotetraploids.

Detailed and critical analysis of morphology of the individual chromosomes in the somatic complements of the diploid as well as tetraploid types has been given for the first time.

Decrease in size of chromosomes with increase in chromosome number was not observed in the case of the mutant studied.

The coefficient of multivalency was found to be fairly high in the mutant which indicated that continuous selection in the selfed progenies would be necessary for some years.

Maximum germination of pollen grain was obtained on 12% sugar solution in the case of tetraploid gram and on 7½% in the case of diploid gram which indicates that the tetraploid gram has higher osmotic pressure indicative of higher concentration of water-soluble cell contents.

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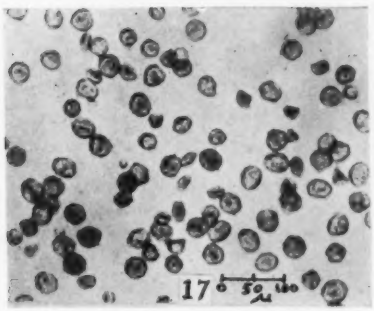
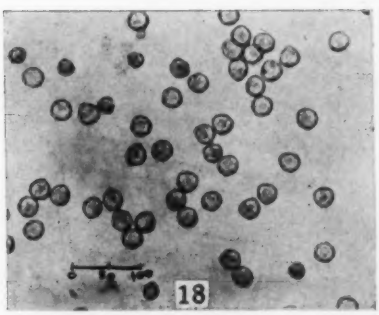
EXPLANATION OF PLATE XI

Figs. 17–18. Photomicrographs. Fig. 17. Pollen size variation in the autotetraploid gram. Magnification, ×100. Fig. 18. Regular sized pollen grains of the diploid Chafa gram. Magnification, ×1000.


17


18

STUDIES ON THE PHYTOTOXIC ACTION OF BHC & DDT ON THE COMMON CUCURBITACEOUS PLANTS IN WEST BENGAL

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Introduction

The family Cucurbitaceæ yields several vegetables which are very popular and widely cultivated throughout West Bengal. Unfortunately, they are subjected to the attack of some notorious insect pests like *Aulachophora* spp. and *Epilachna* spp., etc., which cause extensive damage each year. A suitable control of the cucurbit pests was a problem in the past. The crude indigenous method of dusting the foliage with cowdung ash was pitiably inadequate to combat large outbreaks of the pests. Pyrethrum product on the other hand had the disadvantage of being costly without any residual action.

Soon after their introduction, BHC and DDT insecticides gained wide popularity in the State for their remarkable efficacy in controlling a wide range of insect pests and their safe and easy handling. The residual action of DDT and the low price of structure of BHC, made them favourites of the cultivators. Unfortunately the problem of the control of the cucurbit pests remained the same, as DDT and BHC, though effective against the pests, were reported to have phytotoxic action on the cucurbitaceous plants. Workers like Underhill (1945), Dudley (1947) and Tet *et al.* (1948) reported DDT to be phytotoxic to cucurbits, while samething was reported about BHC by Stoker (1948) and Crowell and Morrison (1950). These adverse reports about BHC and DDT prevented the authors from suggesting their use to the cultivators as they were not ready to undertake the risk without proper investigation of the problem.

As regards the cause of the phytotoxic action of BHC and DDT, workers varied widely in their views. Thus Carruth and Harvey (1947), Carruth and Howe (1948) and Alban and Keirns (1948) maintained that impurities and isomer other than para isomer were largely responsible for the phytotoxic action of DDT. Similarly, in case of BHC, isomer and impurities other than gamma isomer, were attributed to the cause of the foliage injury.

DDT dust, had been applied to the assorted varieties of cucurbits at Corvallis in Western Orgeon in 1944 and 1945 without evidence of foliage injury. During 1946, reports of DDT injury to cucurbits were received from workers in a number of Eastern States. These differences in results were then attributed to the differences in climatic condition (Crowell and Morrison, 1950).

In 1947 it was accidentally learnt that the presence of moisture on the foliage at the time of treatment could make the organic insecticides phytotoxic to the cucurbits. This was confirmed in 1948. It is not known whether or not the very small water-soluble fraction of the insecticides is the cause of plant injury (Crowell and Morrison, 1950).

Considering these controversies, the authors felt the need of a proper trial of BHC and DDT under the climatic conditions of West Bengal which differs much from that of other-countries, where the above observations were made. The authors want to make it clear that the primary object of this experiment was to find out whether 5% BHC dust and 0·1% DDT spray could be tolerated by the common cucurbit plants as the above concentration of BHC and DDT had proved quite effective against *Aulachophora* spp. and *Epilachna* spp.

MATERIAL AND METHODS

The cucurbit varieties which have been tested during the period under observation in West Bengal are listed below. Besides the botanical names the local names and the corresponding English names are furnished.

Botanical Name	Local name and the corresponding available English name
1. *Cucurbita pepo*	.. Kumra, Pumpkin.
2. *Lagenaria vulgaris*	.. Lau, Gourd.
3. *Luffa acutangula*	.. Jhinga, Ribbed gourd.
4. *L. ægyptiaca*	.. Dhudul, Spongy gourd.
5. *Cucumis sativus*	.. Sasha, Cucumber.
6. *Memordica indica*	.. Uchay, Bitter gourd.
7. *Citrullus colocynthis*	.. Kharmuja, Melon.
8. *C. vulgaris*	.. Tarmuja, Water melon.
9. *Trichosanthis dioica*	.. Patal.

The insecticides selected for the study were:—

(1) BHC 5% dust containing 0·65 gamma isomer in talc. (supplied by both M/s. Imperial Chemical Industries and Gladstone Lyall & Co.).

(2) BHC 4% dust prepared by mixing road dust with BHC 5% dust in talc.

(3) DDT 0·1% spray prepared @ 2 lb. of DDT 50% in talc. (Geigy-Guesarol—550) in 100 gallons of water.

(4) DDT 0·5% spray prepared @ 1 lb. of DDT 50% in talc. (Geigy-Guesarol—550) in 100 gallons of water.

Two strengths of benzene hexachloride were used with a view that if 5% BHC acted positively in that case, action of BHC 4% would be noticed to find out whether it acted in either way. Similarly two strengths of DDT were used with that in view. In West Bengal, the cultivators generally use BHC as dust and DDT as spray. That is why only the dust formulation of BHC and spray formulations of DDT were tried in this experiment.

All the treatments were applied both in green house and in field conditions. Plants of different ages ranging from 7–10 days' plants to well grown-up plants were used. For dusting both shaking gently through muslin bags and small type of hand dusters (Roonwell) were used. In case of spraying, automiser and shavol sprayer were used. The whole experiment lasted from July 1953 to September 1954, covering almost all the atmospheric conditions of West Bengal.

In the green house condition 10 earthen pots were prepared for each variety of plants. Six seeds were sown, at a time in each pot. After germination, the best 2 plants were kept for the experiment and others were up-rooted. All the pots were treated at a time. For each variety 4 pots were treated with the scheduled insecticide leaving one as control or check. The same arrangement was made for other 5 pots of the same variety and placed in a randomised way (*i.e.*, replicated twice).

Detailed observation on the phytotoxic action on plants were taken on every 24 hours after treatment, continuing upto 3 days in the first phase. In the second phase the observation continued on every 7 days after treatment upto a month (Tables I-V). Plants were however under observation until they met their natural death in the long run. Simultaneously the average maximum and minimum temperature and the percentage of humidity for a period of 15 days ranging from 7 days before and 7 days after the treatment were recorded to see whether meteorological factors had any hand in it. The experiment was repeated twice or thrice in the same year.

TABLE I

Treatment given :— BHC 5% dust. Verieties tried :—*Cucurbita pepo, Luffa acutangula, L. ægyptiaca, Cucumis sativus, Memordica indica, Citrullus colocynthis, C. vulgaris, Trichosanthis dioica*

Age of the plants treated (in weeks)	Any phytotoxic action observed on plants after						Date of application	Fortnightly average		
	24 hrs.	48 hrs.	72 hrs.	One week	Two weeks	One month		Max. temp. °F.	Min. temp. °F	Humidity per cent.
One ..	Nil	Nil	Nil	Nil	Nil	Nil	12—8—53	91·8	89·9	83·4
Three ..	Nil	Nil	Nil	Nil	Nil	Nil	31—7—53	91·8	88·8	85·8
Seven ..	Nil	Nil	Nil	Nil	Nil	Nil	17—11—53	85·2	82·4	82·0
Nine ..	Nil	Nil	Nil	Nil	Nil	Nil	7—1—54	82·1	75·1	66·9
Fruiting stage ..	Nil	Nil	Nil	Nil	Nil	Nil	17—12—53	84·7	78·7	73·8

TABLE II

Treatment given :— BHC 5% dust. Veriety tried :—*Lagenaria vulgaris* (Lau.)

Age of the plants treated (in weeks)	Any phytotoxic action observed on plants after						Date of application	Fortnightly average		
	24 hrs.	48 hrs.	72 hrs.	One week	Two weeks	One month		Max. temp. °F.	Min. temp. °F.	Humidity per cent.
One ..	Burning patches appeared on leaves	Upper portion of the shoot found drooping with burning effect	All leaves showed burning patches. Leaves found crumpling with edges upside down	Almost died	Died	..	12—8—53	91·8	89·9	83·4
Three ..	do	do	do	do	do	..	31—7—53	91·8	88·8	85·8
Seven ..	do	do	do	do	do	..	17—11—53	85·2	82·4	82·0
Nine ..	Nothing	Burning in patches appeared on leaves	Upper portion of the shoot found drooping with burning effect	All leaves showed burning patches. Leaves fonnd crumpling with edges upside down	Almost died	Almost died	7—1—54	82·1	75·1	66·9
Fruiting stage	do	do	do	do	do	do	17—12—53	84·7	78·7	73·8

TABLE III

Treatment given : BHC 4% dust. Varieties tried :—*Cucurbita pepo, Luffa acutangula, L. ægyptiaca, Cucumis sativus, Memordica indica, Citrullus colocynthis, C. vulgaris, Trichosanthis dioica*

Age of the plants treated (in weeks)	Any phytotoxic action observed on plants after						Date of application	Fortnightly average		
	24 hrs.	48 hrs.	72 hrs.	One week	Two weeks	One month		Max. temp. °F.	Min. temp. °F.	Humidity per cent.
One ..	Nil	Nil	Nil	Nil	Nil	Nil	12—8–53	91·8	89·9	83·4
Three ..	Nil	Nil	Nil	Nil	Nil	Nil	37—1–53	91·8	88·8	85·8
Seven ..	Nil	Nil	Nil	Nil	Nil	Nil	17–11–53	85·2	82·4	82·0
Nine ..	Nil	Nil	Nil	Nil	Nil	Nil	7—1–54	82·1	75·1	66·9
Fruiting stage ..	Nil	Nil	Nil	Nil	Nil	Nil	17–12–53	84·7	78·7	73·8

TABLE IV

Treatment given :— BHC 4% dust. Variety tried :—*Lagenaria vulgaris* (Lau.)

Age of the plants treated (in weeks)	Any phytotoxic action observed on plants after						Date of application	Fortnightly average		
	24 hrs.	48 hrs.	72 hrs.	One week	Two weeks	One month		Max. temp. °F.	Min. temp. °F.	Humidity per cent.
One ..	Burning patches appeared in less No. of leaves than BHC 5%	Upper portion of the shoot found drooping with burning effect	Almost all leaves showed burning patches. Leaves found crumpling with edges upside down	About to die	Almost died	Almost dead	12—8—53	91·8	89·9	83·4
Three ..	do	do	do	do	do	do	31—7—53	91·8	88·8	85·8
Seven ..	do	do	do	do	do	do	17–11–53	85·2	82·4	82·0
Nine ..	Nothing	Burning patches appeared in less No. of leaves than BHC 5%	Upper portion of the shoot found drooping with burning effect	Almost all leaves showed burning patches. Leaves found crumpling with edges upside down	About to die	Almost dying	7—1—54	82·1	75·1	66·9
Fruiting stage	do	do	do	do	do	do	17–12–53	84·7	78·7	73·8

TABLE V

Treatment given :— DDT 0·1% spray. Varieties tried :—*Cucurbita pepo, Luffa acutangula, L. aegyptiaca, Cucumis sativus, Memordica indica, Citrullus colocynthis, C. vulgaris, Trichosanthis dioica, Lagenaria vulgaris*

Age of the plants treated (in weeks)	Any phytotoxic action observed on plants after						Date of application	Fortnightly average		
	24 hrs.	48 hrs.	72 hrs.	One week	Two weeks	One month		Max. temp. ° F.	Min. temp. ° F.	Humidity per cent.
One ..	Nil	Nil	Nil	Nil	Nil	Nil	12- 8-53	91·8	89·9	83·4
Three ..	Nil	Nil	Nil	Nil	Nil	Ni	31- 7-53	91·8	88·8	85·8
Seven ..	Nil	Nil	Nil	Nil	Nil	Nil	17-11-53	85·2	82·4	82·0
Nine ..	Nil	Nil	Nil	Nil	Nil	Nil	7- 1-54	82·1	75·1	66·9
Fruiting stage ..	Nil	Nil	Nil	Nil	Nil	Nil	17-12-53	84·7	78·7	73·8

N.B.—All the trials with the same results were repeated under different weather conditions, as will be evidenced from the different date of experiment and their fortnightly average max. temp., min. temp. and percentage of humidity.

Date of experiment	Fortnightly average		
	Max. temp. ° F.	Min. temp. ° F.	Humidity per cent.
3-11-53	92·5	87·0	80·7
6- 3-54	89·0	86·0	88·5
12- 5-54	99·0	80·2	60·0
23- 7-54	91·9	88·7	84·2

In the field condition almost same procedure was followed. The insecticides were tried in the Agricultural Farm of the Institute. They were also tried in Agricultural Farm of Ramakrishna Sangha at Dakshineswar in 24-Parganas and at Badkulla in Nadia Dist.

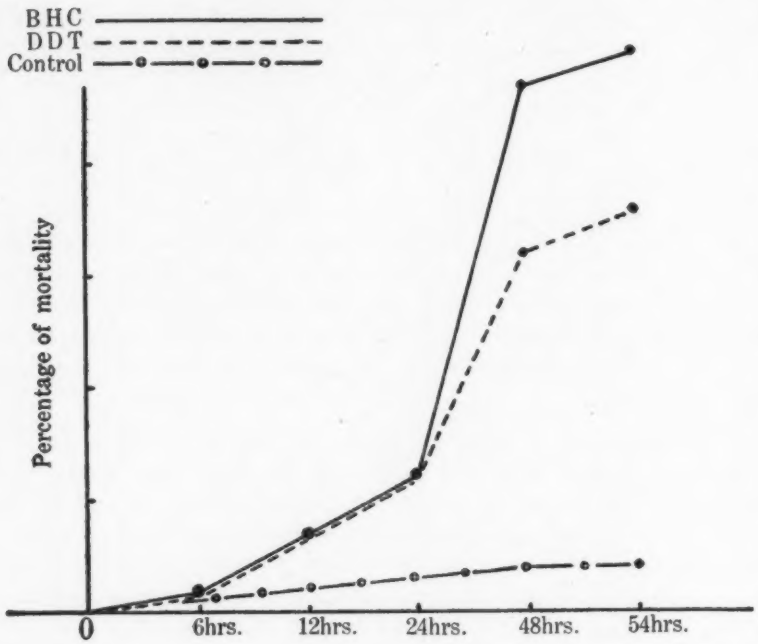
Five plants of the same age (grown-up plants) were selected for each of the variety of plants concerned. All the plants selected were treated with our scheduled insecticide @ 10 lb. per acre in case of dust and @ 50 gallons per acre in case of spraying. Whole experiment were replicated twice. Casual observation on the phytotoxic effect was taken on every 24 hours upto 3 days whereas detailed observations were taken after 7 days of treatments. Observation lasted upto a period of 1 month after treatment (Tables I–V). Plant were however under observation until they met their natural death.

To be perfectly sure that the prescribed treatments might serve the purpose of controlling the pest, a small-scale experiment was carried out in the laboratory at the end. Nine twigs of *Luffa ægyptiaca* (Dhundul), (preferred much by *Epilachna* spp.) with 2 leaves were collected, the cut ends of which were dipped in 9 small reagent bottles filled with water. Each such bottle was placed inside a big glass jar separately. First 3 twigs were dusted uniformly with BHC 5%, the second 3 were thoroughly sprayed with DDT 0·1%, last 3 were left as it was *i.e.*, as control or check. Ten *Epilachna* spp. (of the same age) which were kept fasting on the previous day were released in the twigs of each jar. The mouth of each jar was then covered and tied with a thin muslin. Average mortality of the beetle on every hour upto 12 hours and then on every 24 hours till all the insects of any of the treated jars died completely, were recorded (Table VI and the graph).

TABLE VI

Showing average percentage of mortality of *Epilachna* spp. after use of insecticides on their food on :—

Treatment	6 hrs.	12 hrs.	24 hrs.	48 hrs.	54 hrs.	Remarks
Control ..	Nil	Nil	Nil	06·66	06·66	Mortality started within five hours of treatment of DDT. Action of BHC started within 6 hours of treatment. Mortality completed within 54 hours of treatment of BHC and within 60 hrs. of treatment of DDT, while mortality in the control was about 6% during that period.
BHC 5% dust ..	03·33	13·33	23·33	93·33	100·00	
DDT 0·1% spray	03·33	13·33	23·33	63·33	70·00	



GRAPH

Dusted leaves were however dried up earlier than other. It was perhaps due to dusting on wet leaves. Leaves remained wet due to rain (Crowell and Morrison, 1950).

NATURE OF PHYTOTOXIC INJURY

Insecticidal injury consisted of 2 types, burning and chlorosis (yellowing of the leaves). Leaf-burn occurred in round patches within 24 hours in case of 1–7 weeks plants, *e.g.*, *Lagenaria vulgaris* (Table II) and within 48 hours in case of 9 weeks to grown-up plants, *e.g.*, *L. vulgaris* (Tables II and IV) after application and resulted in necrosis of all parts of the affected leaves. After appearance of burning patches the leaves found drooping down with burning effect and ultimately died after being charred, *e.g.*, *L. vulgaris* (Tables II and IV). Chlorosis often required several weeks in which to appear and usually did not lead to necrosis.

RESULTS

As the results were almost identical in case of all other cucurbitaceous plants than *L. vulgaris* for a particular treatment, the results were summarised

in 2 tables for each treatment. It should be borne in mind that the observation on plants left as control or check were always included in the first table of each treatment as the results were almost same in both the cases (*i.e.*, in treated and in control). As all the varieties behaved same in case of DDT treatment the results of the same treatment were summarised in a single table (Table V). Treatment of DDT 0·05% sol. was not included in the table as the higher dose of DDT, *i.e.*, 0·1%, did not show any phytotoxic action on plants.

Both BHC 5% and 4% were found to have no appreciable phytotoxic action on all the 9 selected cucurbits except *L. vulgaris* (Tables I and III). In case of *L. vulgaris* phytotoxicity of BHC 5% and BHC 4% appeared in round burning patches on leaves. Difference between BHC 5% and BHC 4% were—burning patches appeared in less number of leaves than BHC 5% (Tables II and IV). DDT were found to have no appreciable phytotoxic action on any of the 9 selected cucurbitaceous plants (Table V).

From the Table VI it appeared that mortality of *Epilachna* spp. started within 5 hours of treatments of DDT 0·1%. In case of BHC 5%, mortality started within 6 hours of treatments. Mortality completed within 54 hours of BHC treatment and within 60 hours of DDT treatment (Graph 1).

Discussion and Practical Significance of the Result

In summing up the result it can be suggested that except one, all the other vegetably grown cucurbits (of course those which were under trial) can stand BHC 5% dust in West Bengal condition, without being inflicted with any appreciable foliage injury upon themselves. Only plant which is particularly susceptible to BHC injury is *Lagenaria vulgaris* (Lau, gourd). It cannot stand BHC, perhaps, in any form. BHC 4% dust produced the same result. It can however stand DDT 0·1% spray very successfully. This infact helped in enhancing the production (eye observation).

Unlike Alban and Keirns (1948), Carruth and Howe (1948), Carruth and Harvy (1947), neither the impurities, which were said to be present in these formulations, nor the climatic condition as observed at Corvallis in Western Orgeon from 1944–46 were found responsible for the phytotoxic injury on the cucurbits like *Cucurbita pepo* (Kumra), *Luffa acutangula* (Jhinga), *L. ægyptiaca* (Dhudul), *Cucumis sativus* (Sasha), *Memordica indica* (Uchay), *Citrullus colocynthis* (Kharmuja), *C. vulgaris* (Tarmuja) and *Trichosanthis dioica* (Patal) in West Bengal. The climate of West Bengal appeared to be congenial for the treatment of these insecticides as it did no.

seem to influence any phytotoxic injury on plant foliage. All the selected cucurbits could however withstand DDT 0·1% spray.

Plants like *Cucurbita pepo* (Kumra), *Luffa acutangula* (Jhinga), *Luffa ægyptiaca* (Dhundul), *Cucumis sativus* (Sasha), *Memordica indica* (Uchay), *Citrullus colocynthis* (Kharmuja), *Citrullus vulgaris* (Tarmuja) and *Trichosanthis dioica* (Patal) can be both dusted with BHC 5% (Gammexane and Hexidole) and sprayed with DDT 0·1%. To be doubly sure one must dust the plants in such a way as to make a thin covering of the insecticide on the foliage. For the kitchen garden use, the insecticide may be dusted by shaking muslin bag filled with insecticide in the direction of wind so that the wind may cover the plant uniformly. DDT should be sprayed @ 2 lb. of DDT 50% (Guesarol-550) in talc. in 100 gallons of water. The ordinary hand sprayer or hand-made syringe may be used for the purpose.

One must be very careful not to use these insecticides when the leaf surfaces of the plant remain wet as it might in some cases, influence phytotoxic action on cucurbits (*vide* mortality experiment of *Epilachna* spp. in Dhundul).

In case of *Lagenaria vulgaris* (Lau.), DDT @ 2 lb. of DDT 50% (Guesarol-550) in 100 gallons of water can safely be sprayed without any foliage injury. All these doses are strong enough to repeal insect attack (Table VI). BHC in any form is strongly discouraged for use on *L. vulgaris* (Lau.). The use of BHC will surely spoil the crop totally.

Some may argue that the insecticides like pyrethrum products and others which have no phytotoxic action on cucurbits can easily be used on *L. vulgaris* when BHC produces adverse effect on this plant. To us the argument does not seem to be wise as DDT has some definite advantages over the pyrethrum products. The use of DDT is economical from the point of both its price structure and its power of retention of toxicity to the insect for a much longer period. It not only helps in eradicating the pest without any foliage injury but adds to the plants' growth. More precisely plants are doubly blessed with the use of DDT, *i.e.*, control of insect and at the same time more production.

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